

Airway Inflammation Before and After Bronchial Thermoplasty in Severe Asthma

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Background: Bronchial thermoplasty (BT) is a bronchoscopic treatment for severe asthma, of which the working mechanism and responder profile are partly unknown. The aim of this study is to analyse whether BT alters airway inflammation by epithelial gene expression, inflammatory cell counts and cytokines, and whether this relates to treatment response.

Methods: In this clinical trial, 28 severe asthma patients underwent bronchoscopy before and after treatment to obtain bronchial brushes and bronchoalveolar lavage fluid (BALF) from treated and untreated airways. RNA was extracted from bronchial brushes for transcriptome analysis, and BALF cells and cytokines were analysed. Asthma quality of life questionnaires were used to distinguish responders from non-responders. We compared results before and after treatment, between treated and untreated airways, and between responders and non-responders.

Results: Gene expression of airway epithelium related to airway inflammation gene set was significantly downregulated in treated airways compared to untreated airways, although this did not differ for patients before and after treatment. No differences were observed in cell counts and cytokines, neither from the untreated compared to treated airways, nor before and after treatment. At baseline, compared to non-responders, the expression of genes related to glycolysis in bronchial epithelium was downregulated and both BALF and blood eosinophil counts were higher in responders.

Conclusion: Local differences in gene sets pertaining to epithelial inflammatory status were identified between treated and untreated airways after treatment, not resulting in changes in differential cell counts and cytokine analyses in BALF. Secondly, baseline epithelial glycolysis genes and eosinophil counts in BALF and blood were different between responders and non-responders. The observations from this study demonstrate the potential impact of BT on epithelial gene expression related to airway inflammation while also identifying a possible responder profile.

Keywords: airway inflammation, bronchial thermoplasty, bronchoalveolar lavage fluid cells, cytokines, epithelial transcriptome

Introduction

Asthma is a heterogeneous disease with episodes of dyspnoea due to bronchial hyperreactivity. The heterogeneity is reflected by varying degrees of chronic airway inflammation and tissue remodeling, and differences in response to treatment.¹ Mild-to-moderate asthmatic patients are usually well controlled by inhaled medication, while approximately 5% of the asthma patients remain unstable despite high-dose inhaled medication and/or oral corticosteroid treatment.² Alternative therapeutic options for these severe asthma patients predominantly target type-2 immune responses, such as anti-immunoglobulin E (IgE), anti-interleukin (IL)-5/anti-IL-5R and anti-interleukin-4R treatment.³⁻⁶ A notable number

of severe asthma patients, however, are not eligible for type-2 inflammation targeted therapies or do not respond clinically to these treatments.⁷ Therefore, there is a need for other targeted therapies for these patients.

A treatment developed specifically to target airway remodeling is bronchial thermoplasty (BT). During this bronchoscopic treatment, medium- to large-sized airways are heated by radiofrequency energy.⁸ Several randomized studies and large “real world” registries have shown clinical benefit after BT, with improvements in quality of life, asthma control and reduction of exacerbations, hospitalizations and emergency department visits.^{9–12} BT treatment has been designed to reduce airway smooth muscle (ASM) mass, which is known to be increased in asthma patients and, importantly, correlates with asthma severity.¹³ However, so far most studies have not found a correlation between the reduction of ASM mass after BT^{14–16} and treatment response, as measured by asthma control and quality of life questionnaires.^{17–19} Therefore, the exact mechanism of action and responder profile of BT remains still incompletely understood.²⁰

Besides ASM cells, bronchial epithelial cells are also exposed to the radiofrequency energy during BT, which has led to disruption of the epithelium immediately after BT.^{21,22} The impact of BT on the epithelium also leads to higher epithelial expression of MUC5AC and higher levels of IL-13R α 2 and IFN- α / β .²³ In addition, both ASM and bronchial epithelial cells can produce pro-inflammatory mediators in severe asthma patients.^{13,24–28} We hypothesize that BT alters airway inflammation as detected by epithelial gene expression, inflammatory cell counts and cytokines and this modification correlates with clinical response. Hence, this study aims to assess the impact of BT treatment on 1) the transcriptome of bronchial epithelium and 2) analyse inflammatory cytokines and cell counts in the bronchoalveolar lavage fluid (BALF). Furthermore, we investigated whether the observed changes are related to treatment response.

Materials and Methods

Subjects and Study Design

This study is a sub-study of the TASMA trial (ClinicalTrials.gov NCT02225392) in which severe asthma patients are treated with BT.¹⁹ Patients who fulfilled the World Health Organization²⁹ and Innovative Medicine Initiative³⁰ definitions were included after written informed consent. Patients were recruited in 2 centers in the Netherlands (Amsterdam University Medical Centers and University Medical Center in Groningen) and 2 centers in the United Kingdom (Royal Brompton Hospital and the Chelsea & Westminster Hospital, both in London). This study was performed in compliance with the Declaration of Helsinki. Ethical approval was provided by the ethical committees of the 4 centers. The study design of this sub-study is shown in Figure 1. Baseline data collected 3 weeks before BT treatment was used for analysis. This included a bronchoscopy performed to obtain bronchial brushes and BALF from the left lower lobe and lingula, respectively. Six months after BT, all patients underwent a post-BT bronchoscopy to obtain brushes and BALF from the left lower lobe and lingula airways again and, in addition, brushes and BALF were obtained from the untreated middle lobe.

Bronchial Thermoplasty and Bronchoscopies

Bronchoscopies were performed under conscious sedation (remifentanyl/propofol)³¹ or general anaesthesia according to the current recommendations^{8,32} using the Alair System (Boston Scientific, USA). Per protocol, the middle lobe was not treated by BT and served as a control. Patients were treated with prednisolone 50 mg starting 3 days before the procedure, on the day itself and one day thereafter. During each bronchoscopy, BALF was performed in wedge position in the lingula using 4–5 fractions of 20 mL of saline with a minimum of 15–20 mL recovery. Thereafter, endobronchial brushes

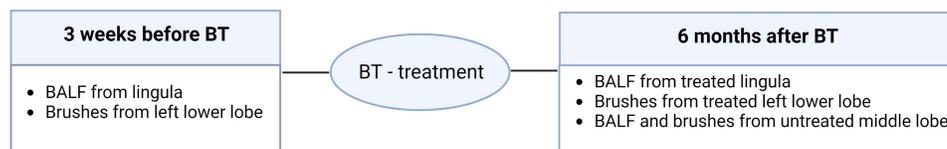


Figure 1 Study design. Three weeks before BT treatment baseline data were collected: BALF from the lingula and brushes from the left lower lobe. Six months after BT treatment BALF and brushes were collected from the treated lingula and left lower lobe, and untreated middle lobe. (Figure created with BioRender.com).

Abbreviations: BT, bronchial thermoplasty; BALF, bronchoalveolar lavage fluid.

were taken from the left lower lobe. During follow-up research bronchoscopy, 6 months after BT treatment, BALF and brushes were collected from the treated lingual and left lower lobe airways and untreated middle lobe. Per bronchoscopy 4 brushes were obtained from the left lower lobe of which 2 brushes were pooled for RNA analysis. In the middle lobe, one brush was used for RNA analysis.

RNA Isolation, Sequencing and Analysis

Bronchial epithelial cells were the predominant cells (>95%) obtained from the bronchial brushings. If available 2 brushes were pooled together and pelleted by centrifugation for 10 minutes at 267g at 4°C. TRIzol™ (Thermo Fisher Scientific, Paisley, United Kingdom) was added to the cell pellet and subsequently stored at –80°C until RNA isolation. The RNA isolation method, cDNA library preparation, RNA sequencing and analysis are provided in Appendix 1. For heat maps, log₂ gene expression values were normalized with Z-scores calculated by $(X (\text{value of the individual sample}) - \mu (\text{average of the row})) / \sigma (\text{standard deviation of the row})$. The clustering of genes represented in heat maps was based on k-means clustering. The combination of Z-scores for all genes are represented below the heat maps in red and blue, red indicating high expression and blue for low expression.

Gene Set Enrichment Analysis (GSEA)

All differentially expressed genes with a q-value less than 0.05 compared between 2 groups were used for gene set enrichment analysis (GSEA) in the online platform (www.gsea-msigdb.org). The gene identifiers were uploaded in the molecular signature database of the website, and the overlapping gene sets were computed. The P-values were derived on the basis of hypergeometric distribution for $k - 1$, K , $N - K$, and n , where k is the number of genes in the intersection of the query set with a set from the Molecular Signature Database, K is the number of genes in the set from the Molecular Signature Database, N is the total number of all human genes, and n is the number of genes in the query set. The false discovery rate (q-value) analog of the hypergeometric P-value was determined by correction for multiple hypothesis testing according to Benjamini and Hochberg. The gene sets thus identified were plotted in heat maps to visualize gene expression of all individuals separately.

Broncho Alveolar Lavage Fluid (BALF) Processing

BALF was centrifuged at 267g for 10 minutes at 4°C and brake at 3. The supernatant was collected and stored at –80°C in aliquots until analyses were performed. The cells in the pellet were resuspended in phosphate buffered saline and counted using a Burker-Turk hemocytometer (Emergo, Landsmeer, The Netherlands). When available, 500 cells were differentially counted on Diff-Quik stained cytospin slides as eosinophils, neutrophils, macrophages/monocytes, lymphocytes and epithelial (columnar and squamous) cells. The following mediators were assessed in BALF by Luminex: CXCL-8 (IL-8), CXCL-10 (IP-10), IL-6, G-CSF, GM-CSF, IL-1 α , IL-1 β , TNF- α , IL-17A, GRO- α , IL-5, IL-13, IL-4, IL-33, TSLP, IL-12p70, IL-18, Eotaxin 2/3 and VEGF-a (CXCL-8, CXCL-10 and MIF using BioRad reagents and the others using Invitrogen reagents). Myeloperoxidase (MPO) for neutrophil activation was determined by ELISA and eosinophil cationic protein (ECP) for eosinophil activation by Mesoscale.

Statistical Analysis

No sample size calculation was performed for this explorative study. Statistical analyses were performed in GraphPad Prism version 5.01 (GraphPad Software Inc) or IBM SPSS Statistics version 25.0. Demographic parameters were provided as mean with SD or median with interquartile ranges. Differences in cell counts and cytokines between pre-BT, the untreated middle lobe and BT-treated lobes were analysed using Friedman test for paired non-parametric analyses. The AQLQ score was chosen to assess quality of life, in line with previous research.¹⁰ An improvement of ≥ 0.5 points represented a clinically relevant result.³³ Mann–Whitney *U*-test was used to compare responders and non-responders at baseline. One patient was not able to fill in the questionnaires due to high symptom burden after BT and was therefore judged a non-responder by the treating physician. Two-sided P-values were used with a statistical significance at $P < 0.05$.

Results

Twenty-eight severe asthma patients were included in this study, a subgroup from the TASMA trial.¹⁹ One patient was excluded from analysis due to anti-IL-5 treatment during the follow-up period. Paired bronchial epithelial samples for transcriptome analysis were available for 25 patients (2 samples were too low in RNA quality for adequate sequencing and therefore excluded) and paired BALF samples were available from 26 patients (in a single patient BALF recovery was insufficient after BT). Baseline characteristics are shown in Table 1. Overall, Asthma Quality of Life Questionnaire (AQLQ) and 6-item Asthma Control Questionnaire (ACQ-6) significantly improved after BT: AQLQ increased from 4.13 (± 0.94) to 4.84 (± 1.20) (P-value = 0.004, n = 26) and ACQ-6 decreased from 2.65 (± 0.6) to 2.14 (± 1.0) (P-value = 0.01, n = 26). Thirteen patients had an improvement of ≥ 0.5 on their AQLQ scores after BT therapy and were defined as responders. Responders showed a clinically relevant mean change in AQLQ of 1.7 (± 0.6), while AQLQ scores in non-responders had a non-relevant change of -0.3 (± 0.5). The exacerbation rate per half year significantly reduced from 2.48 (± 2.6) prior to BT to 0.59 (± 1.05) after BT (P-value = 0.0004, n = 27). The exacerbation rate in the responder group per 6 months significantly reduced from 2.81 (± 3.3) prior to BT to 0.31 (± 0.48) after BT (P-value = 0.02, n = 13).

The impact of BT treatment after 6 months follow-up was assessed by RNA-sequencing of brushed bronchial epithelial cells from BT-treated airways compared with that of untreated airways as control. Five hundred twelve genes were differentially expressed in brushes of the bronchial epithelium comparing the untreated middle lobe and BT-treated airways at the same time point. GSEA showed that these genes belong to TNF- α signalling via NF κ B, myc targets, hypoxia, UV response, P53, metabolic genes (oxidative phosphorylation, glycolysis, fatty acid metabolism) and apoptosis genes (Table 2).

The top gene set pertaining to inflammatory status (TNF- α signalling via NF κ B), as shown in heat maps (Figure 2A), was significantly downregulated in bronchial epithelium from BT-treated airways compared to untreated airways, obtained at the same time point. The line graphs plotting the Z-scores, combined for all genes displayed in the heat maps, also showed a significant decrease of TNF- α signalling via NF κ B for BT-treated airways compared to untreated airways (Figure 2B). The transcriptome of bronchial epithelial brushes obtained 3 weeks before BT treatment, ie at baseline, revealed no differences in gene expression as compared to 6 months after BT treatment (Figure 2C), although

Table 1 Patient Characteristics at Baseline

| Characteristics | Data |
|---|-----------------------------|
| Number of patients | 28 |
| Sex (male/female) | 6/22 |
| Age (years) | 45 (± 12) |
| BMI (kg/m ²) | 28.2 (± 4.8) |
| Dose of LABA (μ g/day salmeterol equivalents) | 136 (± 35) |
| Dose of ICS (μ g/day fluticasone equivalents) | 1158 (± 501) |
| Number of patients on maintenance use of OCS | 9 |
| Dose of oral prednisone (mg/day) in OCS users | 13 (± 6) |
| Number of patients on omalizumab | 3 |
| Pre-short-acting bronchodilator FEV1 (% predicted) | 86 (± 24) |
| Post-short-acting bronchodilator FEV1 (% predicted) | 100 (± 19) |
| FEV1 reversibility (%) | 14 (± 15) |
| PC20 (mg/mL) | 0.35 (0.03–2.92 IQR) (N=27) |
| FeNO | 17.5 (12.8–41.8 IQR) (N=25) |
| Number of patients with a positive skin prick test | 15 (54%) |
| Total serum IgE (kU/L) | 51.95 (17.05–210.25 IQR) |
| Blood eosinophil count (10 ⁹ /L) | 0.14 (0.06–0.29 IQR) |

Notes: Data are presented as absolute numbers, mean \pm standard deviation) or median with interquartile range (IQR).

Abbreviations: BMI, body mass index; LABA, long-acting β_2 -agonist; ICS, Inhaled corticosteroids; OCS, oral corticosteroids; FEV1, forced expiratory volume in 1 second; PC20, methacholine provocation test; FeNO, fraction exhaled nitric oxide; IgE, Immunoglobulin E.

Table 2 Gene Set Enrichment Analysis (GSEA) Showing the List of Differentially Expressed Gene Sets

| Gene Set Name [# Genes (K)] | Description | # Genes in Overlap (k) | p-value | FDR q-value |
|--|--|------------------------|------------------------|------------------------|
| HALLMARK_TNFA_SIGNALING_VIA_NFKB [200] | Genes regulated by NG-kB in response to TNF [GeneID=7124]. | 37 | 2.61×10^{-37} | 1.31×10^{-35} |
| HALLMARK_MYC_TARGETS_V1 [200] | A subgroup of genes regulated by MYC - version 1 (v1). | 24 | 5.56×10^{-20} | 1.41×10^{-18} |
| HALLMARK_HYPOXIA [200] | Genes up-regulated in response to low oxygen levels (hypoxia). | 18 | 4.06×10^{-13} | 6.77×10^{-12} |
| HALLMARK_UV_RESPONSE_UP [158] | Genes up-regulated in response to ultraviolet (UV) radiation. | 14 | 2.07×10^{-10} | 2.59×10^{-9} |
| HALLMARK_P53_PATHWAY [200] | Genes involved in p53 pathways and networks. | 15 | 4.9×10^{-10} | 4.9×10^{-9} |
| HALLMARK_OXIDATIVE_PHOSPHORYLATION [200] | Genes encoding proteins involved in oxidative phosphorylation. | 13 | 3.9×10^{-8} | 3.25×10^{-7} |
| HALLMARK_APOPTOSIS [161] | Genes mediating programmed cell death (apoptosis) by activation of caspases. | 11 | 2.63×10^{-7} | 1.88×10^{-6} |
| HALLMARK_GLYCOLYSIS [200] | Genes encoding proteins involved in glycolysis and gluconeogenesis. | 12 | 3.09×10^{-7} | 1.93×10^{-6} |
| HALLMARK_FATTY_ACID_METABOLISM [158] | Genes encoding proteins involved in metabolism of fatty acids. | 10 | 1.85×10^{-6} | 1.03×10^{-5} |
| HALLMARK_ALLOGRAFT_REJECTION [200] | Genes up-regulates during transplant rejection. | 10 | 1.49×10^{-5} | 5.74×10^{-5} |

the variability in gene expression was less after BT as reflected by its standard deviations (pre BT 0.6 SD; post-BT region 0.43 SD).

The responders and non-responders did not show differences in overall gene expression in bronchial epithelium from the treated and untreated airways, ie after correction for multiple testing (Figure 3A). However, when analysing potential candidate gene sets at baseline, genes representing glycolysis (Figure 3B) and corticosteroid response genes (Supplementary Figure 1A) were found to be potential predictors of response to BT treatment. At baseline, heatmaps of genes representing glycolysis are reduced in responders and heatmaps representing corticosteroid response genes are enhanced in the responders, compared to that in non-responders. Line graphs representing Z-scores of all genes in the gene set showed significant increases in glycolysis genes in non-responders compared to responders (Figure 3C). Similar comparisons showed reduced expression of corticosteroid response genes in non-responders as compared to responders, despite the use of (inhaled) corticosteroids. However, this was not significant (Supplementary Figure 1B).

No significant differences were found in differentiated cell counts in BALF before and after BT (Supplementary Figure 2). In addition, no differences between untreated middle lobe BALF samples were detected as compared to treated airways (Supplementary Figure 3). Neither did we find significant changes in blood eosinophils between baseline ($0.13 \times 10^9/L$ (0.06; 0.26 IQR)) compared to post BT values ($0.14 \times 10^9/L$ (0.07; 0.22 IQR), P-value = 0.95).

When comparing responders with non-responders at baseline, a higher BALF eosinophil count was found in responder patients: $0.80 \times 10^9/L$ (0.20; 2.15 IQR) compared to $0.20 \times 10^9/L$ (0; 0.45 IQR) in non-responders (P-value = 0.02).

In addition, compared to non-responders, it was found that the responders have significant higher blood eosinophil counts ($0.22 \times 10^9/L$ (0.12; 0.30 IQR)) vs ($0.1 \times 10^9/L$ (0.05; 0.12 IQR), P-value = 0.006) and a significant higher total IgE serum count (182 kU/L (436.5; 16.4 IQR) vs 25 kU/L (220; 14.8 IQR), P-value = 0.02). The differences found at baseline between the groups are shown in Figure 4. No other significant differences were found in BALF and blood

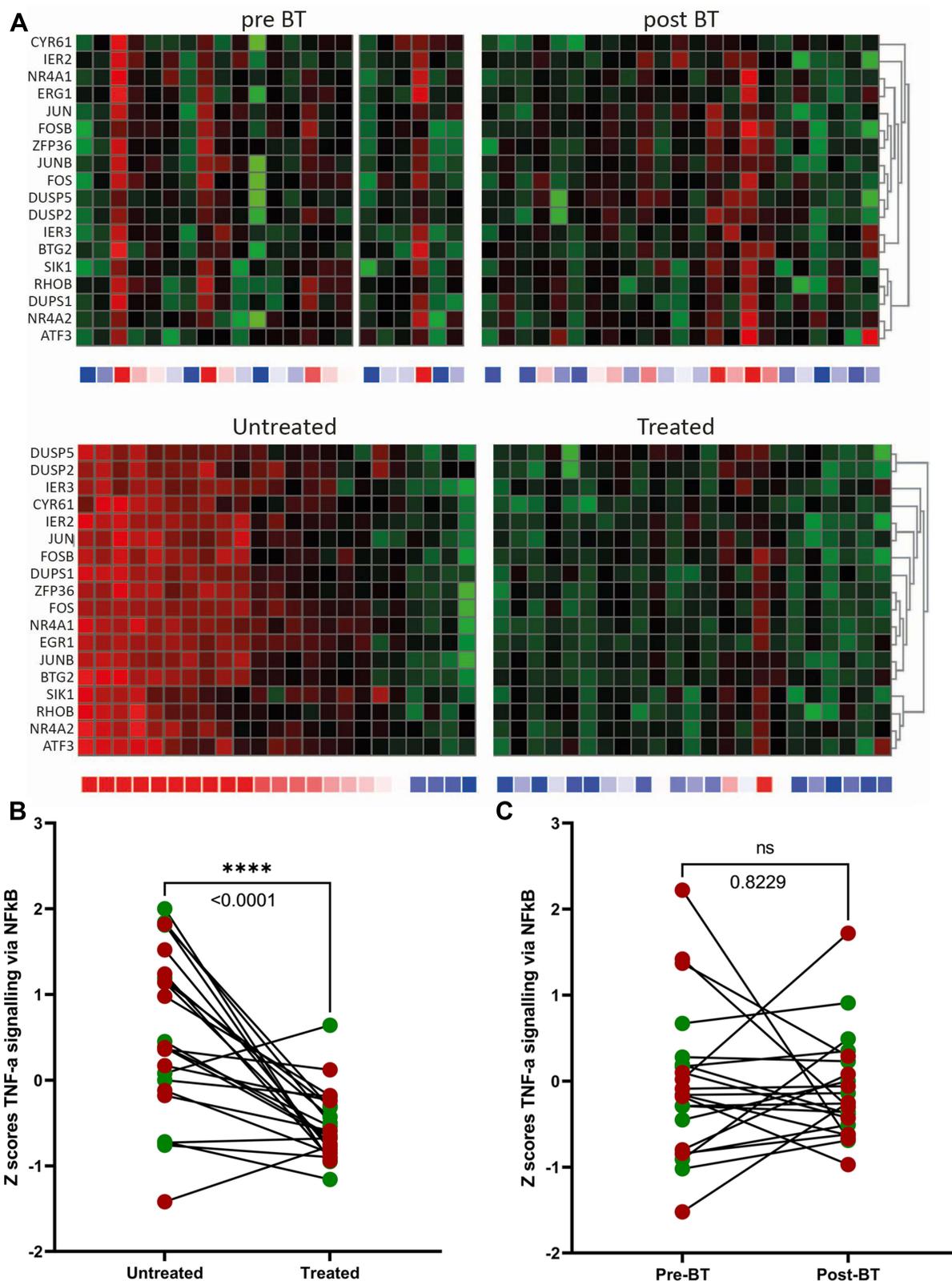


Figure 2 Differentially expressed gene sets before and after BT treatment. **(A)** (upper panel: baseline (pre BT) versus after treatment (post BT)). Heatmaps of inflammatory genes for bronchial epithelial cells showing a non-significant difference between the baseline (pre BT) and treated airways (post BT). **(A)** (lower panel: untreated versus treated (after treatment)). Heatmaps of inflammatory genes for bronchial epithelial cells after BT showing a significant downregulation of the treated lobe compared to the untreated middle lobe. **(B)** (After treatment). Z-scores from heatmaps showing a significant difference between untreated middle lobe and treated lobes after BT ($p < 0.0001 = ****$). There were no significant differences between responders (green dots) and non-responders (red dots). **(C)** (baseline versus after treatment). Z-scores from heatmaps showing a non-significant difference between the baseline (pre-BT) and treated airways (post-BT).

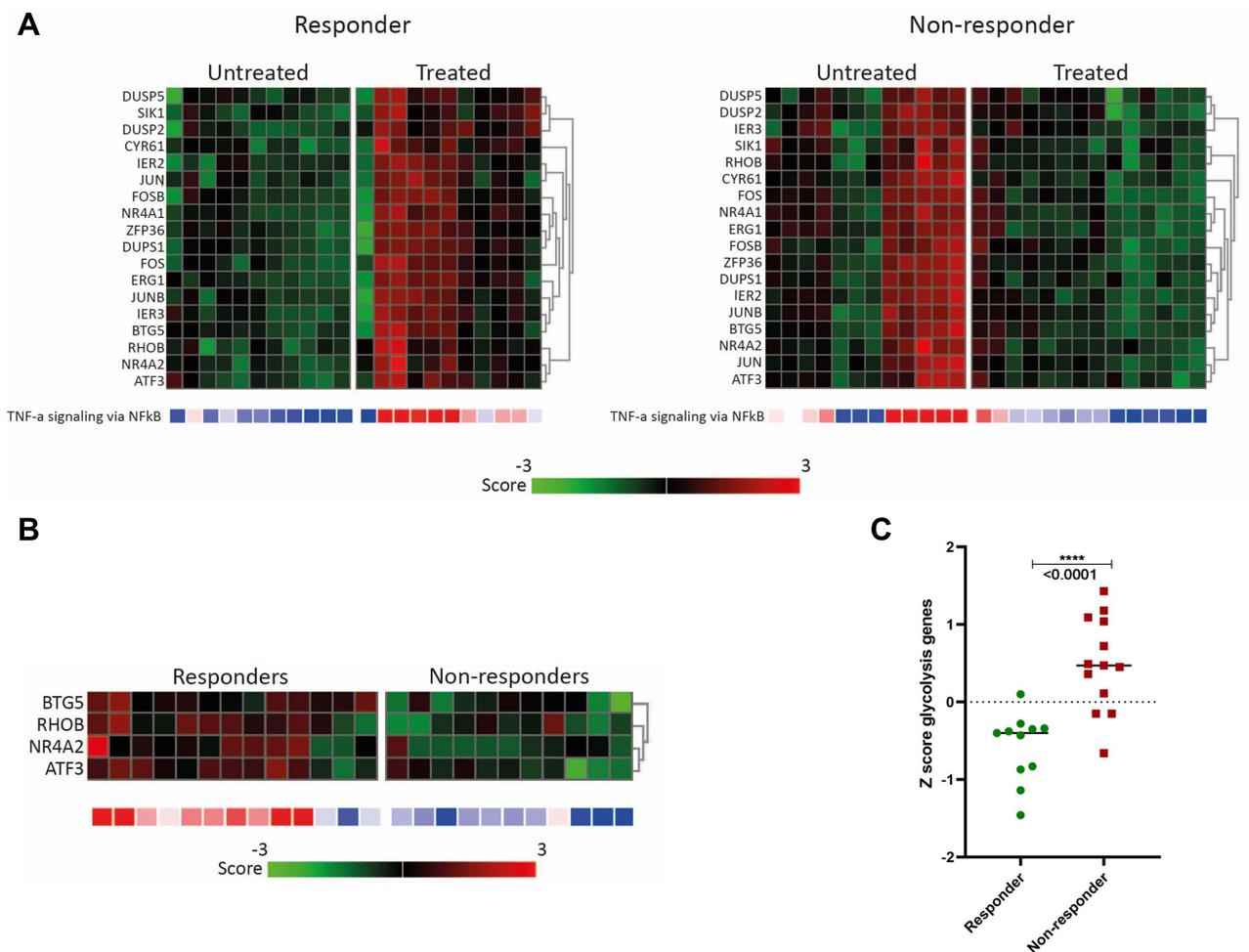


Figure 3 Differentially expressed inflammatory and glycolysis gene sets in the responder and non-responder groups. **(A)** After treatment: Heatmaps of inflammatory genes for bronchial epithelial cells are downregulated in treated airways compared to the untreated (middle) lobe. No differences between responders and non-responders were seen. **(B and C)** at baseline: Glycolysis gene expression in responders and non-responders. **(B)** Heatmaps of genes representing glycolysis showing that glycolysis genes are downregulated in responders. **(C)** Z-scores from glycolysis genes showing a significant difference between responders and non-responders.

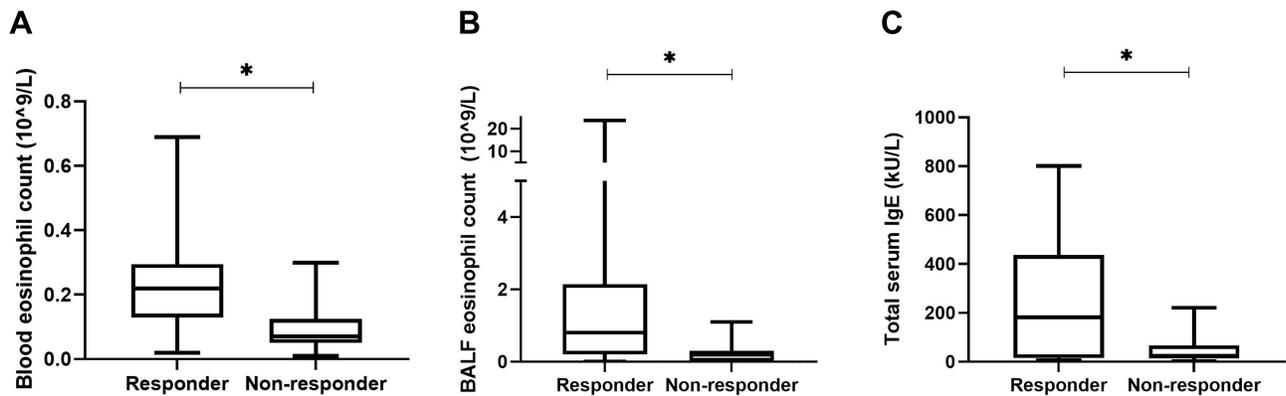


Figure 4 Differences at baseline between responders and non-responders. Mann–Whitney *U*-test analyses showed **(A)** difference in blood eosinophil count ($p=0.006$), **(B)** difference in BALF eosinophil count ($p=0.02$) and **(C)** difference between serum IgE ($p=0.02$) between responders and non-responders. * = $p < 0.05$. **Abbreviations:** AQLQ, asthma quality of life questionnaire; BALF, bronchoalveolar lavage; IgE, Immunoglobulin E.

values between responders and non-responders at baseline ([Supplementary Figure 4](#)). All 3 patients on anti-IgE (Xolair) at baseline were responder.

Only ECP, MPO, VEGF-a, Eotaxin 2/3, GRO- α , CXCL-10, CXCL-8 and MIF were consistently detectable in BALF. There were no differences comparing before with after BT samples nor between the untreated and BT-treated airways ([Supplementary Figures 5](#) and [6](#)), and no significant changes between responders and non-responders ([Supplementary Figure 7](#)).

Discussion

In this study, we show that gene sets pertaining to inflammatory status in bronchial epithelial cells from BT-treated airways were downregulated as compared to untreated airways 6 months after BT. This downregulation was not reflected in an attenuated airway inflammation in BALF, based upon differential cell counts and cytokine analyses. Patients responding to BT treatment were those with enhanced baseline blood and BALF eosinophil counts, despite the use of high levels of (inhaled) corticosteroids. Furthermore, the baseline transcriptome of the bronchial epithelial cells showed that responders had lower expression of genes representing glycolysis.

The first 10 gene sets that are significantly differentially expressed in bronchial epithelial cells between the BT-treated and untreated airways are associated with inflammation and metabolism. It is noteworthy that these differences persist for at least 6 months after treatment, indicating that BT treatment has a long-lasting impact on local cells, which is in line with the reported long-term clinical response 5 to 10 years after treatment.³⁴⁻³⁶

A correlation between airway inflammation and ASM remodeling in asthma is well described.^{37,38} Considering the reduction in ASM after BT and the direct impact of BT on the epithelium, we postulate that the modulation of these BT targets might well have contributed to the effect measured on local inflammatory gene expression changes in the epithelium after BT. Furthermore, from the clinical trials and registries, we know that BT reduces the number of exacerbations, which implies that BT may limit the inflammatory response during exacerbations.^{19,23} On the other hand, it is remarkable that the transcriptome from bronchial epithelial cells taken before and after the treatment were not significantly different and differences in airway inflammation based on cytokines and inflammatory cells in BALF after BT could not be detected. A possible explanation for failing to obtain a significant difference is that the transcriptome at baseline has more variation between patients, whereas after treatment this variation is reduced ([Figure 2B and C](#)). Although patients were requested to keep their baseline asthma medication stable during the TASMA study, it might be that patients have reduced their inhaled corticosteroids (ICS) and oral corticosteroids (OCS) due to better asthma control after BT with less exacerbations. Secondly, the high variation in the middle lobe could potentially be explained by variability of indirect BT treatment effects on the middle lobe like previously reported for ASM^{14,19} and neuroendocrine cells.¹⁷ This, and the fact that the middle lobe remains untreated, might explain local increase in inflammatory gene expression in the middle lobe as opposed to BT-treated airways and the lack of differences in airway inflammation before and after BT. Previous studies on inflammatory cytokines after BT reported conflicting results, where both reductions²³ as no significant changes in cytokines¹⁵ are described. It has to be taken into account that these measurements were done at a different time point compared to our study, with a time window of 3 weeks till 3 months after BT. Colleagues have shown a significant reduction of transforming growth factor beta 1 (TGF- β 1) and chemokine ligand 5 (CCL5) levels after BT in BALF, but no changes in key cytokines such as interleukin (IL)-4, IL-5, IL-13 and IL-17A.^{15,39}

Recently, we reported a defective translational control in bronchial epithelial cells of asthma patients, most pronounced in severe asthma.^{40,41} This leads to enhanced inflammation that correlates with corticosteroid unresponsiveness, neutrophilic inflammation and increased glycolysis with reduced oxidative phosphorylation. Our current data indicates that asthma patients responding to BT treatment had, compared to non-responders, a lower expression of glycolysis genes and higher eosinophilic counts in blood and BALF. Previously, we showed that BT resulted in an increased expression of oxidative phosphorylation genes⁴¹ and others reported an increased expression of glucocorticoid receptors in epithelial cells after BT treatment.⁴² Taken together, these findings are indicative for the apparent normalization of the metabolic function of bronchial epithelial cells by BT treatment ([Figure 5](#)) which links to a distinct BT responder profile.

Responders were defined based on quality of life questionnaires, which is in line with an overall improvement in exacerbation rate. Patients responding to BT treatment in this study, had higher numbers of eosinophils, like previously

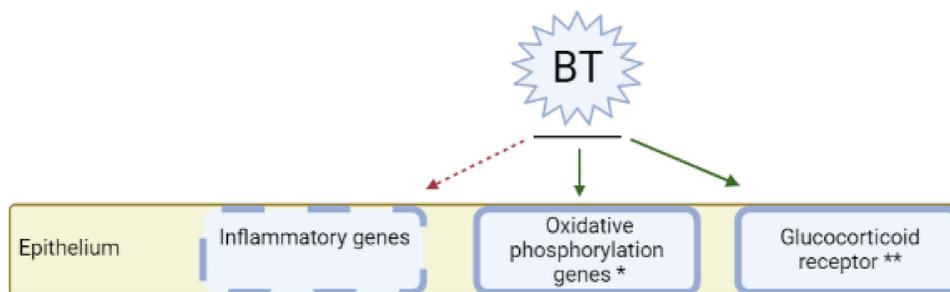


Figure 5 Proposed interaction between BT and inflammation. Inflammatory genes are downregulated after BT, whereas the expression of oxidative phosphorylation genes and glucocorticoid receptors is increased after BT, as described in other studies.^{41,42} *Described in study of Ravi et al⁴¹ **Described in study of Papakonstantinou et al⁴² (Figure created with BioRender.com).

Abbreviation: BT, bronchial thermoplasty.

reported.^{19,23} Among others, our results implicate that severe asthma patients with moderate levels of eosinophils, despite high-level ICS, and/or elevated IgE have a favorable BT response profile. This is in line with the Global Initiative for Asthma guideline, where BT is considered a treatment modality for patients with severe asthma that have no evidence for type-2 inflammation or remain uncontrolled despite type 2-targeted therapy.^{1,20} To distinguish responders from non-responders, next to eosinophils, our results indicate that it might be possible to predict treatment response by using glycolysis gene expression profiles. In a previous report, genes SLPI, MMP3 and MUC19 were identified to be different between responders and non-responders, which we could not confirm for our study.⁴³ Facciolongo et al investigated the expression of inflammation-related genes in BT-treated patients 6 weeks after BT during the 3rd BT treatment. There was no mRNA expression found of IL-4, IL-13 and IL-17A, but a limited expression of IL-5 mRNA in bronchial samples. However, an increase in CD68 transcripts after BT is reported with an association to response.⁴⁴

One of the strengths of this study is that bronchial brushes and BALF were obtained without increasing oral corticosteroids before the bronchoscopy. This is in contrast to other studies in which bronchial samples were generally taken during the BT procedure in conjunction with pre-treatment OCS for 3 days, per treatment protocol.^{8,32} This may attenuate inflammatory parameters and affect transcriptome of bronchial epithelial cells. In addition, there are few studies in which airway inflammation after BT is analyzed in a relatively large patient group like this study.

Some limitations also need to be addressed. BALF and brushes were only obtained from the middle lobe during the follow-up bronchoscopy and not at baseline. This makes it unclear whether the differences found between the BT-treated and untreated airways were induced by BT alone, which need to be investigated in further studies. In addition, it cannot be excluded that some patients did reduce their ICS use while participating in this study, which may have influenced our results. Lastly, we distinguished responders and non-responders 6 months after treatment, whereas the final effects of BT may occur even later.

Conclusion

This study shows local differences in gene sets pertaining to inflammatory status in the epithelium between BT-treated and untreated airways after BT. These differences did not result in changes in differential cell counts and cytokine analyses in BALF. Baseline epithelial glycolysis genes and eosinophil counts in both BALF and blood were different between responders and non-responders. Therefore, BT responders may be distinguished from non-responders at both transcriptome, systemic and local level. In conclusion, this study shows the impact of BT on epithelial expression of genes related to airway inflammation and supports that BT may be most effective in patients with eosinophils despite optimal treatment with corticosteroids.

Abbreviations

ASM, airway smooth muscle; ACQ-6, 6-item asthma control questionnaire; AQLQ, asthma quality of life questionnaire; BALF, bronchoalveolar lavage fluid; BT, bronchial thermoplasty; ECP, eosinophil cationic protein; GSEA, gene set

enrichment analysis; ICS, inhaled corticosteroids; IgE, immunoglobulin E; IL, interleukin; MPO, myeloperoxidase; OCS, oral corticosteroids; SD, standard deviation.

Data Sharing Statement

The datasets generated and/or analysed during the current study are available from the corresponding author upon request.

Clinical Trial Registration Number

ClinicalTrials.gov, NCT02225392

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, drafting the manuscript, revising or critically reviewing the article; gave final approval of the version to be published. All authors have agreed on the journal to which the article has been submitted and agree to be accountable for all aspects of the work.

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Disclosure

René Lutter and Peter I Bonta are co-last authors for this study.

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