

## ORIGINAL ARTICLE Asthma and Rhinitis

# High-affinity immunoglobulin E receptor expression is increased in large and small airways in fatal asthma

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### Summary

**Background** IgE and its high-affinity receptor FcεRI play an important role in allergy and asthma. The distribution of FcεRI expression in the airways and within the airway wall, however, is largely unknown.

**Objective** In this study, we aimed to map the distribution of FcεRI in different layers of large airways (LA) and small airways (SA) in lung tissue from non-smoking and smoking patients who died of asthma [fatal asthma (FA)] and non-smoking controls (CTR).

**Methods** Postmortem lung tissue from 24 cases of non-smoking FA, 13 smoking FA patients and from 19 subjects who died of non-pulmonary causes (CTR) was immunohistochemically stained for FcεRI and AA1 (mast cell tryptase marker). The expression of these markers was analysed in inner, muscle, and outer layers of both LA and SA by image analysis.

**Results** FcεRI expression was higher in non-smoking and smoking FA compared with CTR in the inner and outer layer of SA. In the outer layer of LA, FcεRI expression was higher in non-smoking FA compared with CTR. AA1 was higher in non-smoking FA compared with smoking FA and CTR in the outer layer of the SA, which was correlated with FcεRI in this layer.

**Conclusion** Our results show that the expression of FcεRI is higher in both LA and SA in FA compared with CTR. These differences are predominantly found in the outer layer where they can be attributed in part to the increased mast cell numbers. These results indicate an increased capacity to mount IgE-mediated reactions in FA, both in LA and SA.

**Keywords** fatal asthma, high-affinity IgE receptor (FcεRI), human lung tissue, IgE, mast cells

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### Introduction

Asthma is a chronic inflammatory disease of the airways, which is associated with airway obstruction that is largely reversible. Asthma attacks or exacerbations are predominantly transient, but can be fatal in some cases. Whereas large airways (LA) were first thought to be the major site of inflammation in asthma, it has become clear that inflammatory cells such as eosinophils and lymphocytes are also present in small airways (SA) [1, 2]. Allergen-specific IgE plays an important role in airway inflammation in atopic asthma, and is a determinant of asthma severity as shown in several studies [3, 4].

High levels of IgE increase the surface expression of the high-affinity IgE receptor (FcεRI) by blocking endocytosis

[5, 6]. In addition, cytokines may increase FcεRI expression [7, 8]. This indicates that IgE levels and allergic inflammation partly determine the level of pulmonary FcεRI expression. Both IgE and its high-affinity IgE receptor, FcεRI, are therefore considered as targets in the treatment of asthma. Indeed, the anti-IgE antibody omalizumab was shown to be effective in reducing the frequency of asthma exacerbations and corticosteroid requirement in moderate-to-severe allergic asthma when used in addition to conventional treatment [9]. Interestingly, anti-IgE treatment resulted in a significant reduction in the number of inflammatory cells in lung tissue, including IgE-bearing cells and cells expressing FcεRI [10, 11].

Initially, it was thought that expression of FcεRI was largely restricted to mast cells and basophils. However, in

the last decade, it has become apparent that FcεRI is also present on eosinophils, platelets, neutrophils and antigen-presenting cells, such as dendritic cells (DC) [12–15]. FcεRI present on mast cells is involved in the acute IgE-mediated allergic reaction: cross-linking of FcεRI by IgE and antigen results in degranulation and release of pre-formed mediators (e.g. histamine), synthesis of arachidonic acid metabolites (prostaglandins and leukotrienes) and the release of cytokines and chemokines. In DC, this receptor may be involved in IgE-mediated allergen uptake and presentation, resulting in allergen focusing and enhanced allergen presentation [16–18].

Whereas the role of FcεRI in IgE-mediated allergic reactions is well known, the distribution of its expression in LA and SA is incompletely studied. Such an insight is important in view of the currently recognized role of small airways pathology and function in asthma, and differential exposure of SA and LA to inhaled allergens. Therefore, not only allergen-specific IgE but also the distribution of FcεRI along the bronchial tree is a likely determinant of the severity of physiological and clinical responses to inhaled allergen in asthma. We hypothesized that FcεRI is elevated throughout the bronchial wall of both LA and SA in patients who died of asthma [fatal asthma (FA)] compared with patients who died of non-pulmonary causes [controls (CTR)]. Therefore, in this study, we mapped the distribution of FcεRI in both SA and LA from cases of FA and CTR. Furthermore, we correlated the expression of FcεRI with mast cells and DC in LA and SA. Because smoking is associated with increased serum IgE levels [19] and increases inflammation in lung tissue of asthmatics [20, 21], we compared FcεRI staining in FA lung tissue from smokers and non-smokers.

## Materials and methods

This study is part of a collaborative study between the Department of Pathology, School of Medicine, São Paulo, and the Department of Pulmonology, Leiden University Medical Center [22]. Use of FA and CTR lung tissue for research purposes has been approved by the São Paulo Medical School Ethical Committee.

### Study population

Lung tissue from 37 subjects who died of an asthma attack (FA) and were autopsied in the autopsy service of the São Paulo Medical School, São Paulo, Brazil, between 1996 and 2005, was included in the study (Table 1). We have described previously part of this FA cohort in other reports [2, 23]. An interview with relatives was undertaken to obtain information concerning main diseases. Inclusion criteria for the FA cohort were (1) a history of asthma and death in a crisis, (2) pathological confirmation of the diagnosis by macro- and microscopic examination at

**Table 1.** Characteristics of non-smoking and smoking fatal asthma (FA) and control subjects (CTR)

|                                           | FA          |             | CTR*        |
|-------------------------------------------|-------------|-------------|-------------|
|                                           | Non-smokers | Smokers     | Non-smokers |
| <i>N</i>                                  | 24          | 13          | 19          |
| Gender (M : F)                            | 8 : 16      | 9 : 4       | 4 : 15      |
| Age at death (mean [SD])                  | 44.6 [17.2] | 43.1 [11.8] | 49.3 [13.6] |
| Smoking (Y : N : ex)                      | 0 : 18 : 6  | 13 : 0 : 0  | 0 : 19 : 0  |
| Pack years (mean [SD])                    | –           | 21.8 [15.8] | –           |
| Duration asthma (mean [SD])               | 25.5 [15.9] | 21.1 [15.5] | –           |
| Asthma onset (early : late <sup>†</sup> ) | 13 : 9      | 7 : 4       | –           |
| Oral corticosteroids (Y : N)              | 3 : 21      | 4 : 9       | –           |
| Inhaled corticosteroids (Y : N)           | 4 : 20      | 1 : 12      | –           |

\*Causes of death of controls were acute myocardial infarction (*n* = 12), cerebral haemorrhage (*n* = 4), pulmonary thromboembolism (*n* = 1), hypovolemic (haemorrhagic) shock (*n* = 1) and mitral valvopathy (*n* = 1).

<sup>†</sup>Early onset of asthma: < 12 years of age, late onset of asthma: > 12 years. *N*, number; M, male; F, female; SD, standard deviation; Y, yes; N, no.

autopsy (lung hyperinflation, mucus hypersecretion, epithelial desquamation, thickening of epithelial basement membrane, hypertrophic submucosal glands, thickening of smooth muscle layer and mucosal inflammation with or without eosinophils) and by an interview and (3) death ascribed to status asthmaticus by the pathologists. Tissue was excluded when histological examination showed signs of any other pulmonary disease. Further clinical data (including smoking history, treatment history and previous hospital admissions) were obtained by questionnaires applied to relatives at their homes [2, 23]. For the current study, 37 FA cases were randomly selected from the FA cohort. Twenty-four of the 37 FA cases were non-smokers or ex-smokers at time of death and 13 FA subjects were current smokers at the time of death (Table 1). Lung tissue from 19 non-smoking subjects who died of non-pulmonary causes, with no previously diagnosed pulmonary diseases, was included in the study as control tissue (CTR) (Table 1) [2, 23]. These subjects did not have any history of asthma and had no indications of pulmonary diseases as assessed by macroscopic and microscopic examination.

### Lung tissue processing

Lung tissue of FA and CTR subjects was obtained within 9–33 h after death. Pulmonary samples from peripheral and central areas of the lung were randomly collected from all patients. Tissue was fixed in 4% formaldehyde for 24 h, routinely processed and paraffin embedded. Sections (4 µm thick) were cut and slides from the blocks were screened for the presence of transversally cut LA and SA

(> or <6 mm perimeter, respectively) by haematoxylin and eosin staining. Stainings were performed on serial sections.

### Immunohistochemistry

Antibodies used for immunohistochemical stainings were (1) mouse anti-FcεRI (Upstate Biotechnology, Charlottesville, VA, USA; dilution 1:1200); (2) mouse anti-AA1 (Dako, Glostrup, Denmark; dilution 1:16 000); and (3) rabbit anti-DC-SIGN (Kindly provided by Prof. Dr Y van Kooyk, VU Medical Center, Amsterdam, the Netherlands; dilution 1:2000). Antibodies were diluted in 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS). PBS/BSA 1% alone was used as a negative CTR for the stainings.

Formalin-fixed, paraffin-embedded sections of lung tissue were deparaffinized. Endogenous peroxidase activity was blocked in 0.3% hydrogen peroxide in methanol solution. Slides were subsequently rehydrated. Different antigen retrieval methods were used for the three antibodies: (1) anti-FcεRI: pepsin antigen retrieval: slides were incubated in 0.4% pepsin in 0.01 N hydrochloric acid for 20 min at 37 °C; (2) anti-AA1: trypsin antigen retrieval: slides were incubated in 0.1% trypsin in 0.05 M Tris buffer/0.1% CaCl<sub>2</sub> for 20 min at 37 °C; and (3) anti-DC-SIGN: citrate antigen retrieval: slides were incubated in 0.01 M citrate buffer (pH 6) for 10 min at 120 °C in an autoclave. After antigen retrieval, antibodies were incubated for 1 h. Slides were subsequently incubated with EnVision System<sup>®</sup> anti-mouse or anti-rabbit horseradish peroxidase (Dako Corporation, Carpinteria, CA, USA). Stainings were visualized with EnVision System<sup>®</sup> NovaRed (Dako Corporation) and counterstained with haematoxylin (Klinipath, Duiven, the Netherlands).

### Image analysis

Cell density for mast cell tryptase (using AA1 antibody) and the percentage stained area for FcεRI and the myeloid DC marker DC-SIGN were assessed by image analysis based on colour thresholds. We preferred to use the percentage stained areas for FcεRI and DC-SIGN because in both stainings, we observed cells with cytoplasmic extensions that could be underscored by conventional cell counting. Measurements were made with the image Pro Plus Software version 4.1 for Windows (Media Cybernetics, Silver Spring, MD, USA) on a PC connected to a digital camera coupled with a light microscope (Leica DMR, Leica Microsystems GmbH, Wetzlar, Germany). Transversally cut LA and SA were analysed. The following airway compartments (as described by Bai et al. [24]) were assessed: (1) inner layer: the area between the epithelial basement membrane and the internal smooth muscle border, including the basement membrane; (2) smooth

muscle layer: layer of smooth muscle cells between internal and external smooth muscle border; and (3) outer layer: area between external smooth muscle border and the external limits of the airway, the alveolar parenchyma. In the LA, the external limit of the airway wall was defined by the parenchyma when cartilage and glands were not present in a given field. When present, these structures were considered as the external limits. For LA, cells were counted in a minimum of 10 randomly selected fields from at least two different airways for each subject at a magnification of ×400. For SA, 10 randomly selected fields from the circumference of at least three airways were analysed at the same magnification. In this study, we used coded tissue samples. All stainings on the coded tissue samples were digitized as coded images. Image analysis was based on colour thresholds, thus avoiding subjectivity.

### Statistical analysis

Statistical analysis was performed with the measurements that were available. Because LA and SA were not always present in the tissue blocks of all subjects, the analyses performed include less than the maximal number of subjects included in the study. The number of airways analysed was especially limited for the LA. Numbers of subjects included in the separate analyses are given in the figure legends.

Statistical analysis was performed with SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Data are presented as individual data points and means. Analysis of the distribution of the data showed that the data were not normally distributed. Therefore, we preferred the use of non-parametric analysis methods. Differences between FcεRI and AA1 between non-smoking FA, smoking FA and CTR were analysed by non-parametric Kruskal–Wallis testing. After Kruskal–Wallis testing, means of the groups were tested pairwise by Mann–Whitney *U*-testing. *P*-values were corrected by the Bonferroni–Holmes method. Correlations were analysed by using the non-parametric Spearman's test. Differences between LA and SA in FcεRI and AA1 were analysed by the non-parametric Wilcoxon signed-rank test for two related samples.

### Results

Characteristics of smoking FA, non-smoking FA and CTR subjects are given in Table 1.

There were few positive cells expressing DC-SIGN in lung tissue of FA and CTR, and no differences were observed among groups (values of DC-SIGN-stained areas in FA and CTR are given in Table 2). No correlations between DC-SIGN expression and AA1 or FcεRI were found.

**Table 2.** Percentage DC-SIGN<sup>+</sup>-stained area in controls, non-smoking fatal asthma and smoking fatal asthma

| Airway size | Layer        | Group  | Mean % DC-SIGN <sup>+</sup> -stained area | SD    |
|-------------|--------------|--------|-------------------------------------------|-------|
| Large       | Inner layer  | CTR    | 0.03                                      | 0.053 |
|             |              | FA-nsm | 0.06                                      | 0.108 |
|             |              | FA-sm  | 0.06                                      | 0.083 |
|             | Muscle layer | CTR    | 0.02                                      | 0.037 |
|             |              | FA-nsm | 0.02                                      | 0.041 |
|             |              | FA-sm  | 0.03                                      | 0.050 |
|             | Outer layer  | CTR    | 0.06                                      | 0.051 |
|             |              | FA-nsm | 0.12                                      | 0.177 |
|             |              | FA-sm  | 0.22                                      | 0.173 |
| Small       | Inner layer  | CTR    | 0.02                                      | 0.036 |
|             |              | FA-nsm | 0.06                                      | 0.133 |
|             |              | FA-sm  | 0.02                                      | 0.081 |
|             | Muscle layer | CTR    | 0.01                                      | 0.013 |
|             |              | FA-nsm | 0.01                                      | 0.014 |
|             |              | FA-sm  | 0.01                                      | 0.012 |
|             | Outer layer  | CTR    | 0.02                                      | 0.011 |
|             |              | FA-nsm | 0.02                                      | 0.015 |
|             |              | FA-sm  | 0.02                                      | 0.021 |

Means and standard deviations (SD) of % DC-SIGN<sup>+</sup> area are given.

CTR, controls; FA-nsm, non-smoking fatal asthma; FA-sm, smoking fatal asthma.

#### *Differences in FcεRI and AA1 staining between smoking fatal asthma, non-smoking fatal asthma and control in large and small airways*

FcεRI and AA1 staining was present in the inner layer, muscle layer and outer layer, both in SA (< 6 mm perimeter) and LA (> 6 mm perimeter) in both asthmatics and CTR. Figure 1 shows micrographs of FcεRI and AA1 stainings.

Expression of FcεRI was higher in non-smoking and smoking FA compared with CTR in the inner and outer layer of SA (Fig. 2). In LA, expression of FcεRI was higher in non-smoking FA compared with CTR in the outer layer ( $P = 0.004$ ). No difference was found between smoking FA and non-smoking FA or CTR in this layer (Fig. 2). AA1 staining was higher in non-smoking FA compared with CTR and smoking FA in the outer layer of SA, although not reaching statistical significance after the Bonferroni-Holmes correction (Fig. 2;  $P = 0.02$ ;  $P = 0.031$ , respectively).

#### *Correlation between FcεRI and AA1 stainings*

FcεRI and AA1 stainings were only correlated in the non-smoking FA group. Correlations were found in the muscle layer of LA and outer layer of SA (Fig. 3). In LA of smoking FA and LA and SA of CTR, no correlations were found.

#### *Distribution of FcεRI and AA1 in large and small airways*

Percentage FcεRI<sup>+</sup>-stained area was higher in SA compared with LA in the outer layer only in the non-smoking

FA group (Fig. 4). There were no differences in this staining between LA and SA in the inner and muscle layer. AA1<sup>+</sup> cell density was higher in SA compared with LA in the inner and outer layers in both the FA groups as well as the CTR group (Fig. 4). Results are summarized in Table 3.

#### *Clinical data*

We analysed whether the heterogeneity in both FA groups could have affected our results, by determining the correlation of clinical characteristics with the expression of FcεRI and AA1. None of the characteristics investigated (age of death, gender, late or early onset of asthma, asthma duration, duration of symptoms before death, oral or inhaled corticosteroid use) was correlated with the expression data (data not shown).

#### *Discussion*

In this study, we show that high-affinity IgE receptor (FcεRI)<sup>+</sup> cells are present in all layers of both LA and SA in FA and CTR. FcεRI expression was higher in (non-)smoking FA compared with CTR in the inner and outer layer of SA. In LA, the expression of FcεRI was higher in non-smoking FA compared with CTR in the outer layer. Among asthmatics, FcεRI expression was higher in certain SA compartments when compared with LA. No differences were observed between FcεRI expression in smokers and non-smokers with asthma in any of the compartments investigated. To our knowledge, this is the first study to map the distribution of high-affinity IgE receptor-bearing cells in the airways. Our data indicate that IgE-mediated inflammatory reactions may occur not only in LA but also in SA.

It was shown previously that inflammation in FA is present in both LA and SA, with increases in eosinophils, B cells, CD3<sup>+</sup> and CD4<sup>+</sup> T cells, and also mast cells compared with CTR [2]. We investigated whether the increase in FcεRI in FA could be attributed to an increase in mast cells, the major cell type expressing FcεRI. We did confirm previous findings from our group and others in smoking and non-smoking individuals and in asthmatics [2, 25, 26] by showing that mast cell numbers are higher in SA in FA and CTR. However, this does not explain our findings of higher FcεRI expression in SA that was only observed in asthmatics. In addition, the observed differences in FcεRI expression between asthmatics and CTR were not reflected by concomitant differences in mast cells. We showed that the number of mast cells is higher in the outer layer of SA in non-smoking FA compared with CTR and smoking FA, although this difference did not reach statistical significance after correction for multiple comparisons. The same trend could be observed in the outer layer of LA. Although not reaching statistical significance in both LA and SA, this might indicate a

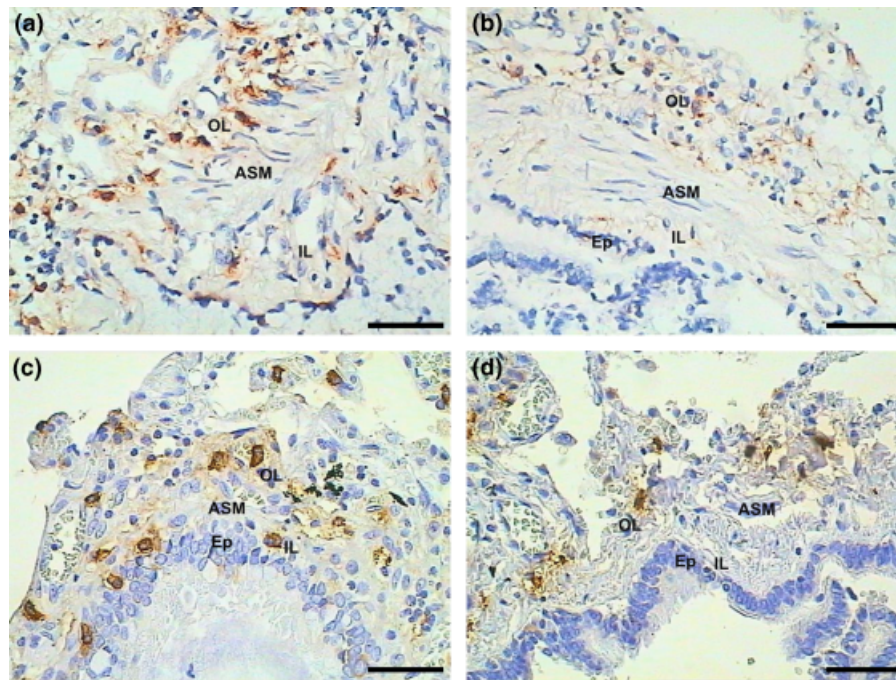


Fig. 1. Photomicrographs of FcεRI and AA1 staining in fatal asthma (FA) and in control (CTR). (a and b) Small airways showing an increased expression of FcεRI (brown) in tissue from a representative non-smoking FA (a) compared with a representative CTR (b) in the inner layer (IL) and outer layer (OL). (c and d) Increased expression of AA1 (brown) in OL of non-smoker FA (c) compared with CTR (d). Positive cells are mainly distributed in outer and inner layer with fewer cells within the airway smooth muscle layer. Scale bar 50 μm. ASM, airway smooth muscle, Ep, epithelium.

suppressive effect of cigarette smoke on mast cells. It has been shown previously that cigarette smoke has a suppressive effect on IgE-mediated degranulation and cytokine release by mouse mast cells *in vitro* [27]. Studies in smoking asthmatics, however, show conflicting results: whereas one study failed to show differences in mast cell numbers in bronchial biopsies between smoking and non-smoking asthmatics [21], another study showed higher numbers in current smokers with asthma compared with non-smoking or ex-smoking asthmatics [28]. Mast cells were also not increased in the muscle layers of LA and SA in FA compared with CTR in our study, which is in contrast to the findings of Brightling et al. [29] in bronchial biopsies from mild-to-moderate asthma but in line with findings by Liesker et al. [30]. Interestingly, correlations between FcεRI expression and mast cells in all compartments investigated were only observed in non-smoking asthma patients in the muscle layer of the LA (where no difference in FcεRI expression or mast cells between asthma and CTR was noted) and in the outer layer of the SA. The correlation between FcεRI and AA1 in the muscle layer suggests that the expression of FcεRI in this layer might be due to the influx of FcεRI-bearing mast cells.

The lack of correlation between FcεRI and mast cells in other layers of the airways indicates that the increased expression of FcεRI in the present study is not simply explained by increases in mast cell numbers, but may indicate regional heterogeneity in FcεRI expression on

mast cells in asthma. Indeed, Andersson et al. [26] showed that the proportion of FcεRI<sup>+</sup> mast cells decreases from LA to SA, whereas the number of mast cells increases. Therefore, higher expression of FcεRI preferentially on mast cells in SA in FA may explain this apparent contradiction. This warrants further investigations, because a high expression of FcεRI on small airways mast cells may make this population more sensitive to allergen exposure.

Our conclusions are based on findings in lung tissue that was collected postmortem. All deaths were confirmed to be caused by an asthma attack (FA) or by non-pulmonary causes (CTR), but we do not have objective detailed information about lung function data or important clinical parameters such as allergy status. However, it needs to be noted that a previous study showed that FcεRI is increased in bronchial biopsies from both atopic and non-atopic asthmatics compared with CTR. Based on available clinical data, including corticosteroid use, we did not find any correlation between these data and the expression of FcεRI and mast cells. Finally, exposure to inhaled particles, such as air pollutants, environmental tobacco smoke or allergens, could have contributed to the heterogeneity observed.

Lack of clinical and lung function data is a major drawback of our postmortem study material. Most of the patients were not receiving hospital care and/or were not adherent/receiving adequate medication, resulting in the lack of this information. However, the use of postmortem

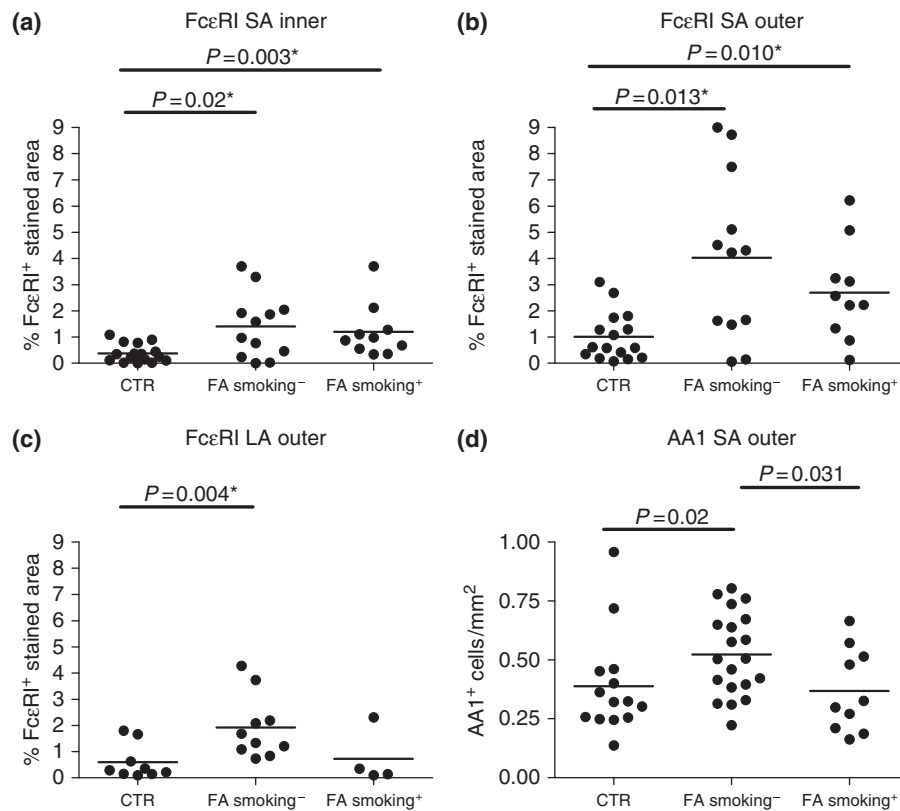


Fig. 2. Increased FcεRI and AA1 staining in non-smoking and smoking fatal asthma (FA) compared with controls (CTR). (a and b) Percentage FcεRI<sup>+</sup>-stained area was higher in (non-) smoking FA compared with CTR in the inner and outer layers of SA ( $n = 16, 10$  and  $12$  for CTR, FA smoking<sup>-</sup> and FA smoking<sup>+</sup>, respectively). (c) In the outer layer of LA, % FcεRI<sup>+</sup>-stained area was higher in non-smoking FA compared with CTR ( $n = 9, 4$  and  $10$  for CTR, FA smoking<sup>-</sup> and FA smoking<sup>+</sup>, respectively). (d) Number of AA1<sup>+</sup> cells was higher in the outer layer of SA in non-smoking FA compared with CTR and smoking FA, but not reaching statistical significance after the Bonferroni–Holmes correction ( $n = 14, 10$  and  $20$  for CTR, FA smoking<sup>-</sup> and FA smoking<sup>+</sup>, respectively). \*Statistical significant after Bonferroni–Holmes correction.

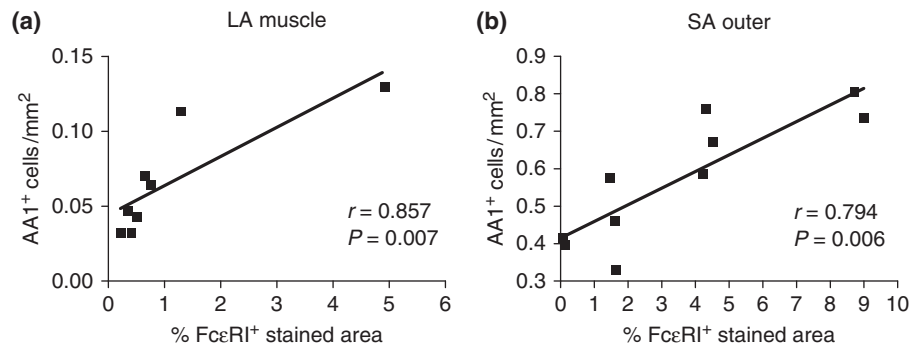


Fig. 3. Correlation between FcεRI and AA1. (a) Correlation between % FcεRI<sup>+</sup>-stained area and the number of AA1<sup>+</sup> cells/mm<sup>2</sup> in the muscle layer of large airways in non-smoking FA ( $n = 8$ ); and (b) correlation in the outer layer of small airways in non-smoking FA ( $n = 10$ ). Graphs show correlation coefficient Spearman's rho ( $r$ ),  $P$ -value and regression line.

tissue samples collected at autopsy is probably be the only feasible way to analyse distal airways and lung parenchyma in asthma. This material has contributed significantly to the characterization of inflammation and structural changes in the lungs in asthma.

Statistical analysis was performed with the measurements that were available for the different layers of LA

and SA. Therefore, the number of cases included in each analysis differed. Differences in the FcεRI between FA and CTR were mainly found in the SA. This may be influenced by a sampling error, which is larger in the analysis of LA than in SA; more SA than LA can be measured and averaged per person. Indeed, the number of subjects who had at least two LA suitable for analysis present in their

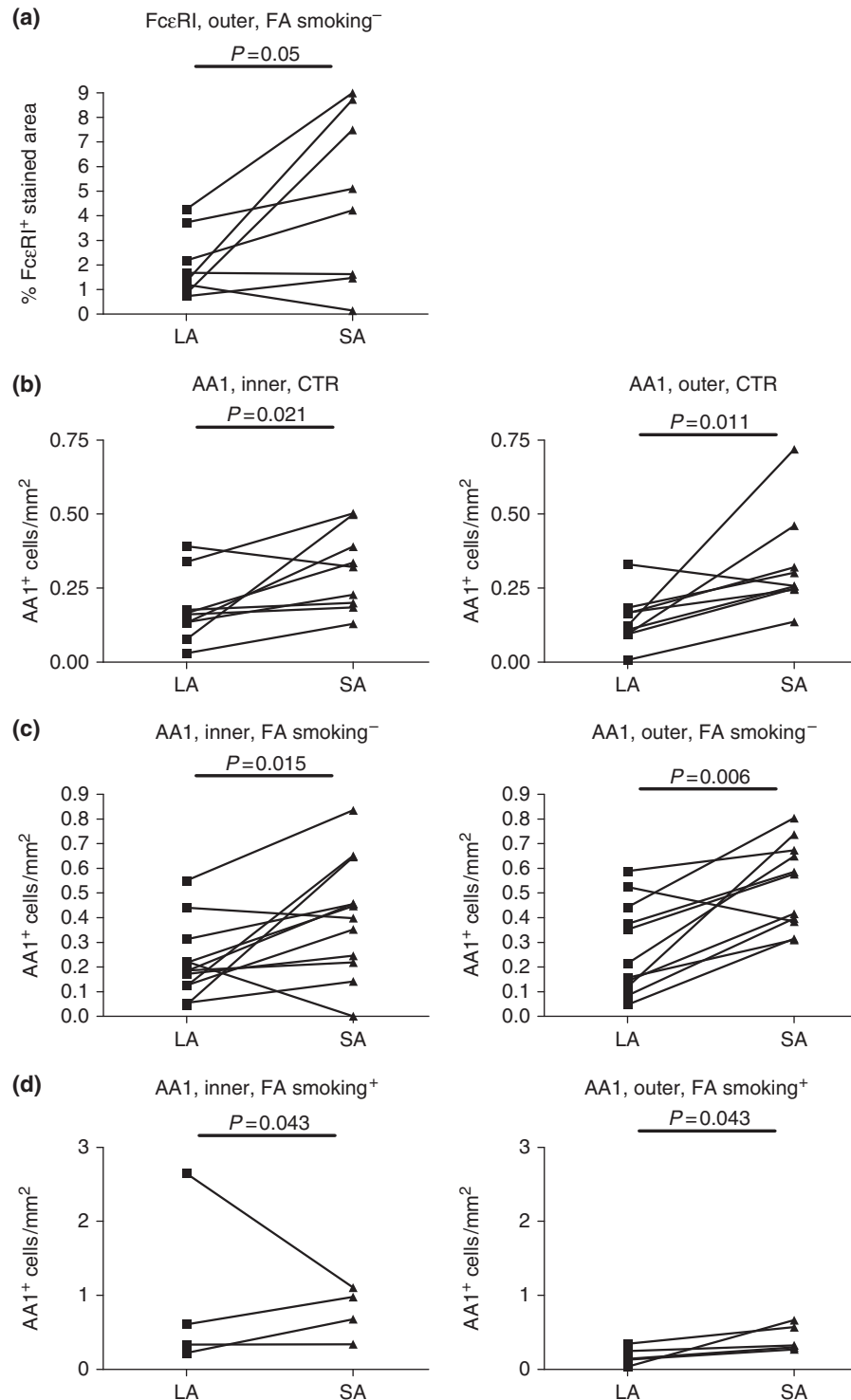


Fig. 4. Difference in FcεRI<sup>+</sup> and AA1<sup>+</sup> cells between large and small airways in FA and CTR. (a) Percentage FcεRI<sup>+</sup>-stained area is higher in SA compared with LA in the outer layer only in non-smoking FA ( $n = 8$ ); (b–d) number of AA1<sup>+</sup> cells/mm<sup>2</sup> is higher in SA compared with LA in both inner ( $n = 9$ ) and outer layer ( $n = 9$ ) in CTR (b), inner ( $n = 12$ ) and outer layer ( $n = 11$ ) in non-smoking FA (c) and inner ( $n = 5$ ) and outer layer ( $n = 5$ ) in smoking FA (d).

tissue blocks was limited. Because data were not normally distributed and sample sizes differed per analysis, we preferred to use non-parametric methods for statistical analysis. Moreover, we prefer to use these methods be-

cause of their conservative nature, leading to more robust and reliable final conclusions. However, we cannot exclude the possibility that we have missed differences due to lack of power and conservative statistical testing.

**Table 3.** Differences in FcεRI and AA1 between small and large airways in controls (CTR), non-smoking fatal asthma (FA-nsm) and smoking fatal asthma (FA-sm)

|       | FA-nsm |     |    | FA-sm |     |    | CTR |     |    |
|-------|--------|-----|----|-------|-----|----|-----|-----|----|
|       | OL     | SML | IL | OL    | SML | IL | OL  | SML | IL |
| FcεRI | ↑      | =   | =  | =     | =   | =  | =   | =   | =  |
| AA1   | ↑      | =   | ↑  | ↑     | =   | ↑  | ↑   | =   | ↑  |

↑, a higher % stained area (FcεRI) and a higher number of AA1<sup>+</sup> cells in SA compared with LA with *P*-value <0.05; =, no difference between LA and SA. FA-nsm, non-smoking fatal asthma; FA-sm, smoking FA; CTR, controls; OL, outer layer; SML, smooth muscle layer; IL, inner layer; SA, small airways; LA, large airways.

We analysed the expression of FcεRI in the three different layers of LA and SA. The inner wall is in close contact with environmental triggers. Inflammatory reactions might therefore be different in this layer compared with the other layers. In the smooth muscle layer, inflammatory cells can have a direct effect on smooth muscle cells, with important functional consequences. We showed that distribution of FcεRI and mast cells within the airway wall is very heterogeneous, which may explain why we observed differences between asthma and CTR only in selected layers of SA or LA. We expected to find the largest differences between FA and CTR in the inner layer of the airways, because this layer is in closest proximity to the environment. However, we also found differences between FA and CTR in the outer layer of the airways. The size of the blood vessels of the outer layer is larger than that of the inner layer [31]. In addition, in the outer layers of especially the SA, the pulmonary circulation contributes to local blood supply, which may have important consequences to inflammatory cell recruitment at this site [32]. Interestingly, we have shown previously that in FA, prominent inflammation and remodelling of extracellular matrix were also observed in this layer of the SA [33].

The enhanced presence of FcεRI in SA in asthma suggests that IgE-mediated reactions to allergens are not limited to the LA but may also occur in the SA when these are exposed to allergens. In line with this, a previous study showed that mast cell-bound IgE is present throughout the airways (LA and SA), but absent in lung parenchyma [34]. Several studies show that allergens may reach the SA, especially when carried by small particles. Pollen allergens from, e.g. birch [35] and grasses [36] are carried by smaller paucimicronic particles (2–5 µm) that reach the SA [37]. House dust mite allergens have also been shown to be present on small (1–5 µm) particles [38], and mouse studies show eosinophil influx in both LA and SA and even alveolar regions of the lungs in mice after intranasal challenge with Der p1 [39].

In summary, our findings on the expression of FcεRI in the SA imply that the IgE-mediated reactions against allergens might also occur at this location, besides the well-established reaction in LA. Inflammation caused by IgE-mediated reactions in SA might contribute to tissue destruction and airway remodelling, which could lead to

small airways dysfunction. Therefore, targeting the activation of FcεRI to treat asthma might dampen IgE-mediated inflammatory reactions and its downstream processes such as airway remodelling in both LA and SA.

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