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## Immune signatures distinguish frequent from non-frequent exacerbators among children with severe asthma

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1 **Immune signatures distinguish frequent from non-frequent exacerbators among children with**  
2 **severe asthma.**

3

4 **To the editor,**

5

6 Asthma is a heterogeneous condition with multiple phenotypes.<sup>1,2</sup> Almost 5% of children with severe  
7 asthma (SA) remain symptomatic despite high doses of inhaled corticosteroids (ICS) and other  
8 controllers.<sup>3</sup> Asthma has long been regarded as a type-2 (T2) disorder, but a dominant Th1 signature,  
9 with Th17 and Th2 cells in a mixed cytokine milieu, was recently described in bronchoalveolar lavages  
10 (BAL) from children with SA.<sup>4</sup> Moreover, among children with SA, cellular components such as IL-17-  
11 producing mucosal-associated invariant T cells may distinguish frequent (FE) from non-frequent  
12 exacerbators (nFE).<sup>5</sup> The inflammatory profiles of children with SA thus appear to be highly complex.  
13 We aimed to provide preliminary results to identify immune signatures associated with clinical  
14 phenotypes of SA.

15 Twenty children with SA were included (**supplementary material & table 1**) and their blood and BAL  
16 collected. FE (n=13) were defined as children with at least three severe exacerbations (SE) in the  
17 previous year and nFE (n=7) as those having one or two.<sup>5</sup> The two groups did not differ, except for the  
18 number of steroid bursts. Samples were also collected from control subjects with non-asthmatic (NA)  
19 severe respiratory conditions (n=10). Preliminary analysis i) confirmed a mixed T1/T2-type cellular  
20 profile in BAL (**figure 1A**), associated with blood eosinophilia (**table 1**), in children with SA relative to  
21 NA, and ii) showed the immune profile of FE to differ from that of nFE (T2 *versus* T1 phenotype;  
22 respectively, **figure 1B&C**). We then performed a comprehensive non-targeted analysis of cytokines  
23 and cells in blood and BAL from children with SA (**supplementary material**) and then constructed a  
24 model to distinguish FE from nFE through supervised analysis (PLS-DA) (**figure 2A**). The model classified  
25 patients with a good predictive value ( $R^2Y=0.799$ ), with both sensitivity and specificity of 100%.  
26 Although all data were used to construct the model, we identified a set of constituents that mostly  
27 supported the differences between the patient groups (**supplementary table 2**, VIP>1). In parallel, we  
28 performed univariate comparisons of each constituent (**supplementary table 2**). Finally, we identified  
29 11 immune constituents to be the most discriminative (PxVIP plotting, VIP>1 and  $p<0.1$ , **figure 2B**). A  
30 higher frequency of ILC1 and Th1-associated chemokines in BAL (CCL2/8, CXCL9-11, **figure 2C&D**)  
31 confirmed the more pronounced T1 phenotype in nFE. Conversely, the local T2 phenotype of FE was  
32 associated with a higher frequency of activated Th17 (IL22<sup>+</sup>) cells in the blood (**figure 2C**) that tended  
33 to correlate with the number of exacerbations ( $\rho=0.41$ ,  $p=0.08$ ). Previous studies found that the  
34 number of circulating Th17 cells was higher in children with moderate-to-severe than mild asthma,<sup>6</sup>

35 that these cells may be steroid resistant,<sup>7</sup> and that PBMC IL-17 secretion was induced by  
36 dexamethasone.<sup>8</sup> Th17 IL-22<sup>+</sup> cells may therefore emerge after repeated systemic steroid  
37 administration received to treat exacerbations, leading to a mixed T2/T17 phenotype. FE also exhibited  
38 lower concentrations of total IgA and higher concentrations of TWEAK, TNFSF14, and TSLP in plasma  
39 (**figure 2E**), and TSLP levels significantly correlated with the number of exacerbations ( $\rho=0.45$ ,  
40  $p=0.045$ ). This is consistent with previous studies showing the involvement of these individual  
41 constituents in the pathogenesis and severity of asthma in children. Plasma constituents, such as  
42 sTNFR1, CCL26, Pentraxin3, CXCL10, IL-32, and sIL16RB, were also highly discriminant, despite high p-  
43 values. All these constituents thus contributed to characterize the FE phenotype and univariate  
44 analysis alone would not have allowed their identification. For example, the T2 chemokine CCL26  
45 tended to be higher in FE and significantly correlated with blood eosinophil counts ( $\rho=0.432$ ,  $p=0.025$ ).  
46 CCL26 is a potent chemoattractant for eosinophils and elevated concentrations of CCL26 in plasma  
47 have been shown to be related to mucosal eosinophilia and the severity of various eosinophilic  
48 disorders (e.g.<sup>9</sup>), which may suggest tissue eosinophilia in FE.

49 In conclusion, despite a small sample size and ICS that may affect the immune response, our study  
50 shows the potential interest of high-dimensional/non-targeted multivariate analysis to identify specific  
51 biological signatures of children with different clinical phenotypes of SA. Although confirmation in an  
52 independent cohort is needed, our study provides new leads for delineating asthma pathogenesis and  
53 identifying new set of targets for diagnosis and personalized treatment.

54

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84 **Author contributions:**

85 GL, KAP, and MLM: designed the research.

86 KAP, MG, BG, CDi, TM, MB, and FM: performed the research.

87 GL, RAT, NG, HF, AN, and CD: were responsible for patient recruitment or establishing the patient  
88 database.

89 KAP and GL: analyzed the data.

90 KAP, GL, and MLM: wrote the manuscript.

91

92 **Abbreviations:** BAL: bronchoalveolar lavage fluids, ICS: inhaled corticosteroids, NA: non-asthmatic,  
93 PLS-DA: partial least squares-discriminant analysis, SA: severe asthma, FE: frequent exacerbators, nFE:  
94 non-frequent exacerbators.

95

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## 104 **Conflict of interests**

105 The authors have no conflict of interests to declare.

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## 108 **Figure legends**

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110 **Figure 1. Complex immune profile of children with different SA phenotypes. A.** Higher frequency of  
111 Th1 cells (trend for Th2) in BAL from children with SA (grey bars) than in that from non-asthmatic  
112 children with severe respiratory disorders (NA, white bars). **B.** The higher frequency of Th1 cells in BAL  
113 of children with SA relative to that in NA children (white bars) was significant only in non-frequent  
114 exacerbators (nFE, n=7; light grey). Conversely, the higher number of Th2 cells in BAL (**B**) and  
115 eosinophils in blood (**C**) was significant only in frequent exacerbators (FE, n=13; dark grey). Data are  
116 shown as box and Tukey whisker plots. P values were obtained using the Kruskal-Wallis test comparing  
117 all groups together.

118

119 **Figure 2. Identification of immune constituents that discriminate between SA children with frequent**  
120 **(FE) and non-frequent (nFE) exacerbations. A.** Graph of the individuals provided by PLS-DA modelling.  
121 **B.** VIP x p values plot of all measured immune constituents and selection of the most discriminating  
122 and significant (red rectangle: VIP>1 and p<0.1; because of the small number of patients in each group,  
123 we tolerated p<0.1 as a cut-off). Identified discriminant immune constituents within cellular (**C**) or  
124 soluble constituents in BAL (**D**) or plasma (**E**), represented as box and Tukey whisker plots. Exact p  
125 values (Mann Whitney test) are indicated.

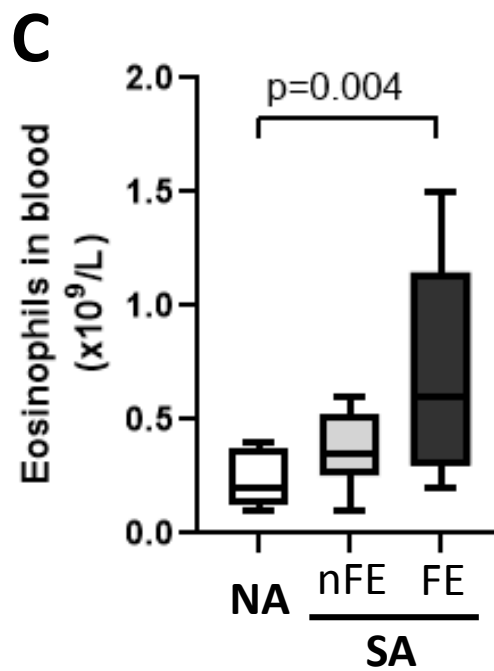
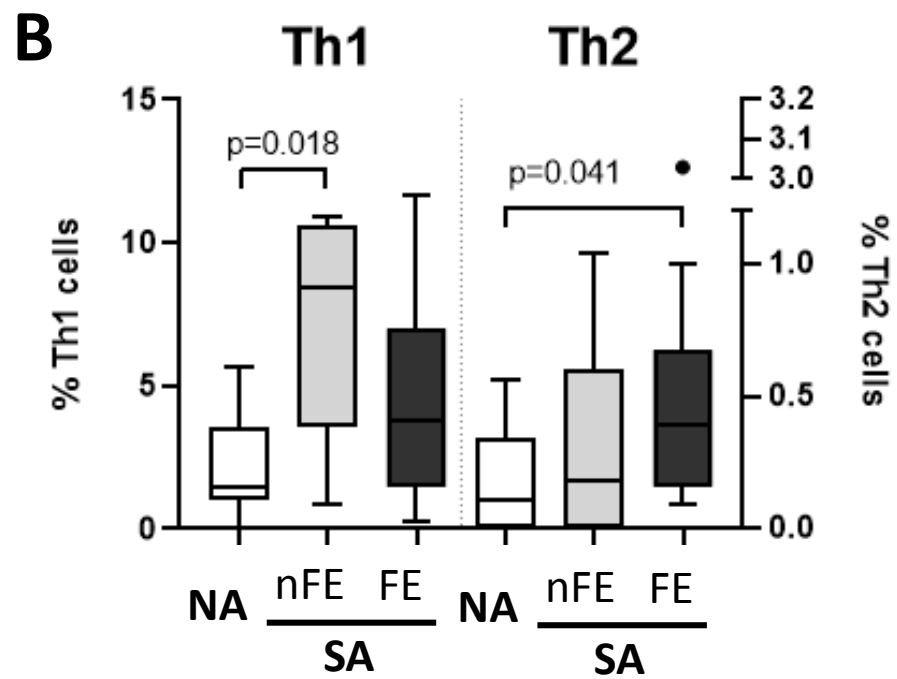
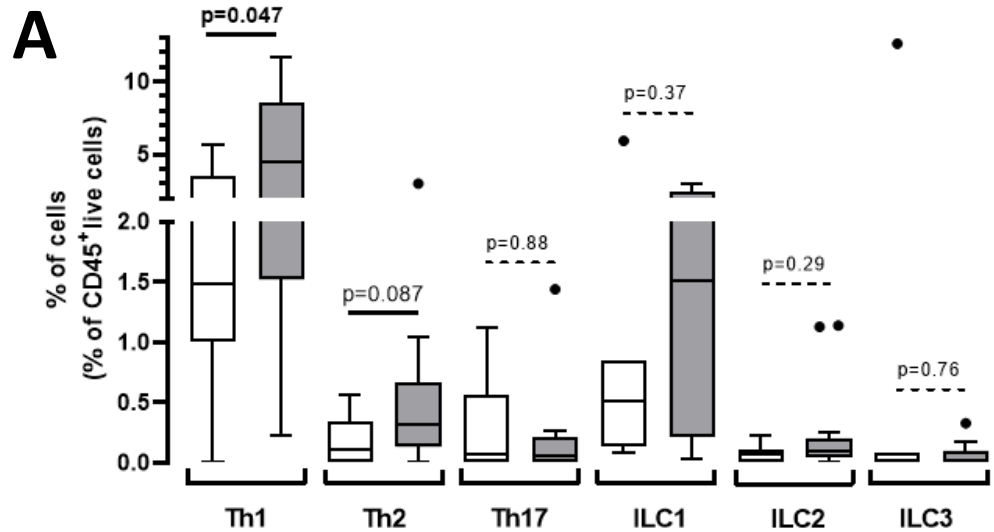
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# Figure 2

