

Sputum inflammatory phenotypes are not stable in children with asthma

Data for on line supplement

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Figure E1 CONSORT diagram, severe asthma cohort**Table E1** Distribution of inflammatory phenotypes for all sputum samples.

| | Severe asthma N=152 | Mild – moderate asthma N=45 | p |
|----------------------------------------------------------|------------------------|-----------------------------------|-------|
| Inflammatory cells, all samples | | | |
| Sputum eosinophils, %, median (IQR) | 3.1 (0.5 – 12.9) | 2.3 (0.5 – 7.7) | 0.228 |
| Sputum neutrophils, %, median (IQR) | 24.8 (11.9 – 52.5) | 26.5 (10.1 – 66.0) | 0.607 |
| Phenotypes, all samples | | | |
| Paucigranulocytic (<i>eos</i> ≤2.5%, <i>neuts</i> ≤54%) | 53 (35%) | 15 (33%) | |
| Eosinophilic (<i>eos</i> >2.5%) | 64 (42%) | 15 (33%) | 0.424 |
| Neutrophilic (<i>neuts</i> >54%) | 17 (11%) | 9 (20%) | |
| Mixed (<i>eos</i> >2.5%; <i>neuts</i> >54%) | 18 (12%) | 6 (13%) | |

Figure E2 Starting inflammatory phenotype and subsequent phenotypes for subjects with severe asthma

Table E2 of the on line supplement, shows the changes in phenotype for both cohorts. For the severe asthma cohort all samples are included in this table (up to maximum of four). In total 22 children (37%) always had the same phenotype and 37 (63%) had a variable phenotype: 17 (29%) changed between one of the inflammatory phenotypes and the paucigranulocytic phenotype and 20 (34%) demonstrated two or more inflammatory phenotypes. In the severe asthma cohort the greater number of sputum samples obtained the more likely the observation of at least one phenotype change. Twenty four subjects produced 4 sputum samples of whom 13 (54%) demonstrated two or more of the inflammatory phenotypes.

Table E2 Longitudinal assessment of sputum inflammatory phenotype for each subject

| | Severe asthma N=42 | Mild-moderate asthma N=17 |
|----------------------------------------------------------------------------------------------|-------------------------------|------------------------------------------|
| Subjects for whom all sputum samples demonstrated the same phenotype | | |
| All paucigranulocytic | 8 (19%) | 4 (24%) |
| All eosinophilic | 5 (12%) | 2 (12%) |
| All neutrophilic | 1 (2%) | 1 (6%) |
| All mixed | 0 | 1 (6%) |
| Total | 14 (33%) | 8 (48%) |
| Subjects demonstrating the paucigranulocytic phenotype and one inflammatory phenotype | | |
| Paucigranulocytic + eosinophilic | 8 (19%) | 0 |
| Paucigranulocytic + neutrophilic | 3 (7%) | 3 (18%) |
| Paucigranulocytic + mixed | 1(2%) | 2 (12%) |
| Total | 12 (29%) | 5 (29%) |
| Subjects demonstrating two or more inflammatory phenotypes | | |
| Eosinophilic + neutrophilic | 3 (7%) | 2 (12%) |
| Eosinophilic + neutrophilic + paucigranulocytic | 1 (2%) | 0 |
| Eosinophilic + mixed | 5 (12%) | 2 (12%) |
| Eosinophilic + mixed + paucigranulocytic | 4 (10%) | 0 |
| Eosinophilic + neutrophilic + mixed | 3 (7%) | 0 |
| Total | 16 (38%) | 4 (24%) |

It is possible that these changes in phenotype may have been as a consequence of very small changes in sputum eosinophils or neutrophils. The eosinophil and neutrophil levels for those children who demonstrated more than one inflammatory phenotype are shown in Figure E2, panels A-E. It can be seen that changes in phenotype are associated with large increases and decreases in sputum eosinophils or neutrophils – not by the levels oscillating around the cut values (2.5% and 54%), represented in each figure by the dotted line.

Figure E3 Changes in sputum eosinophils and neutrophils in subjects demonstrating 2 or more inflammatory phenotypes. A: eosinophilic and neutrophilic phenotypes; B: eosinophilic and neutrophilic and paucigranulocytic phenotypes; C: eosinophilic and mixed phenotypes; D: eosinophilic, mixed and paucigranulocytic phenotypes; E: eosinophilic, neutrophilic and mixed phenotypes. The left hand panel depicts changes in sputum eosinophils and the right hand panel changes in sputum neutrophils. Subjects with mild to moderate asthma are denoted by the suffix M

There were a total of 16 ways in which the sputum phenotype could remain stable or change. In total there were 115 occasions when the sputum inflammatory phenotype was classified at consecutive study visits, as shown in Table E3, on line supplement. On 56 occasions the phenotype remained the same and on the remaining 57 occasions the phenotype changed. The most common changes were from eosinophilic to mixed (13 occasions), from paucigranulocytic to eosinophilic (9 occasions) and from mixed to eosinophilic (9 occasions).

Table E3 Phenotypes of consecutive samples

| | | Phenotype of sample 1 | | | |
|-----------------------|-------------------|------------------------|------------------|--------------|-------------|
| | | Paucigranulocytic c | Eosinophili c | Neutrophilic | Mixed |
| Phenotype of sample 2 | Paucigranulocytic | 26 (4) 23% | 5 (0) 4% | 3 (1) 3% | 3 (1) 3% |
| | Eosinophilic | 9 (0) 8% | 24 (3) 21% | 4 (2) 4% | 9 (0) 8% |
| | Neutrophilic | 4 (2) 4% | 4 (0) 4% | 4 (1) 4% | 1 (0) 1% |
| | Mixed | 1 (0) 1% | 13 (2) 12% | 1 (0) 1% | 2 (1) 2% |

Table E3 Phenotypes of consecutive sputum samples. The shaded boxes represent those occasions when the phenotype remained the same. The totals (%) for both groups are shown; the bracketed figures represent the mild-moderate cohort only.

Figure E4 Scatter plot showing the change in FeNO₅₀ for each phenotype change. The phenotype observed first is shown above the plots and the phenotype observed at the subsequent visit is shown below the x axis. The horizontal bar through each plot represents the group median. Comparison between the groups was made using the Kruskal Wallis test. There was no significant difference between the groups

Figure E5 Scatter plot showing the change in ACT score for each phenotype change. The phenotype observed first is shown above the plots and the phenotype observed at the subsequent visit is shown below the x axis. The horizontal bar through each plot represents

the group median. Comparison between the groups was made using the ANOVA test. There was no significant difference between the groups