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Fluctuation-based clustering reveals phenotypes of patients with different asthma severity

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ABSTRACT Serial peak expiratory flow (PEF) measurements can identify phenotypes in severe adult asthma, enabling more targeted treatment. The feasibility of this approach in children has not been investigated.

Overall, 105 children (67% male, median age 12.4 years) with a range of asthma severities were recruited and followed up over a median of 92 days. PEF was measured twice daily. Fluctuation-based clustering (FBC) was used to identify clusters based on PEF fluctuations. The patients' clinical characteristics were compared between clusters.

Three PEF clusters were identified in 44 children with sufficient measurements. Cluster 1 (27% of patients: n=12) had impaired spirometry (mean forced expiratory volume in 1 s (FEV_1) 71% predicted), significantly higher exhaled nitric oxide (⩾35 ppb) and uncontrolled asthma (asthma control test (ACT) score <20 of 25).

Cluster 2 (45%: n=20) had normal spirometry, the highest proportion of difficult asthma and significantly more patients on a high dose of inhaled corticosteroids (⩾800 µg budesonide).

Cluster 3 (27%: n=12) had mean FEV_1 92% predicted, the highest proportion of patients with no bronchodilator reversibility, a low ICS dose (⩽400 µg budesonide), and controlled asthma (ACT scores ⩾20 of 25).

Three clinically relevant paediatric asthma clusters were identified using FBC analysis on PEF measurements, which could improve telemonitoring diagnostics. The method remains robust even when 80% of measurements were removed. Further research will determine clinical applicability.

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Fluctuation-based clustering is a robust method that identifies clinically relevant subgroups of patients with asthma to refine referral strategies to a tertiary centre https://bit.ly/35g1ldb

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Introduction

Asthma is a heterogenous syndrome with different underlying pathophysiological mechanisms, displaying various symptom profiles and diverse responses to medication [1]. Consequently, the assessment of a patient's individual disease phenotype is crucial for the choice of appropriate treatment [2]. However, the existence of subgroups of asthma patients who do not respond very well to conventional therapy [3] also suggests that currently, some phenotypes may not have been properly identified and characterised, an example being children with therapy-resistant asthma who remain poorly controlled despite high doses of conventional therapy [4]. This group is important as they consume large healthcare resources, and are at greatest risk of severe exacerbations and death [5].

Fluctuations in peak expiratory flow (PEF) values in healthy adults and in patients with stable mild asthma have been found to be significantly related to asthma phenotype [6–8].

In this study we applied a recently developed method of fluctuation-based clustering (FBC) [9] to a prospective observational cohort consisting of children with mild to severe asthma. We hypothesized that applying an observer-independent and data-driven asthma phenotyping methodology solely based on fluctuations of daily PEF might help identify clusters that correspond to clinical phenotypes.

Furthermore, we investigated the extent to which daily PEF measurements entered into a database of FBCs could be used as a low-cost, telemonitoring-based screening, to refine which patients should be referred to a tertiary centre.

Methods

Study population and design

This was a prospective observational cohort study of 120 children, aged 5–17 years, with asthma diagnosed using conventional criteria [10] who were recruited from the Outpatient Department of the Royal Brompton Hospital, London between August 2014 and February 2015. The children were classified into three groups based on treatment levels and previous assessments: severe therapy-resistant asthma (STRA), difficult asthma (DA) and mild-to-moderate asthma (see supplementary Appendix 1) [11]. STRA included children for whom potentially modifiable factors had previously been addressed and who continued to have either or both of ongoing poor control and acute asthma attacks despite receiving high-dose inhaled corticosteroids (ICS) plus add-on therapies (stage 4/5 British Thoracic Society/Scottish Intercollegiate Guidelines Network (BTS/SIGN) guidelines) [12]. DA included children previously or currently prescribed high-dose ICS who had been found to have modifiable factors (such as poor adherence) as a cause for ongoing poor asthma control. Mild-to-moderate asthma included those with well or partly controlled asthma according to Global Initiative for Asthma (GINA) guidelines [13] with a prescribed dose of ICS of ⩽250 µg fluticasone propionate or ⩽400 µg budesonide (or equivalent) per day with the need for none or no more than one controller medication.

After the baseline visit, the patients' daily PEF and adherence to medication was monitored for 2–6 months [14]. The follow-up appointment was combined with the next clinic appointment to minimise hospital visits. The study was approved by the Regional Ethical Committee (NRES Committee London-Westminster). All parents/legal guardians gave written informed consent prior to any study procedures and the children gave age-appropriate assent. The study was registered with clinicaltrials.gov (identifier: NCT02252289).

Daily assessments

PEF measurements

Daily PEF measurements were performed in the morning and evening using a validated electronic peak flow meter (PiKo-1, nSpire Health, Longmont, CO, USA) according to international guidelines [15, 16] (see supplementary material). Data were downloaded at the follow-up visit using an infrared cradle and reviewed using PikoNET software (nSpire Health, Longmont, CO, USA).

Adherence monitoring

Daily adherence was monitored using an electronic monitoring device (Smartinhaler™ Adherium, New Zealand). Smartinhaler devices were available for Symbicort, Seretide and Flixotide. Electronic monitoring devices were attached to the patient's own inhaler and contained a microchip that recorded the date and time the device was activated. We explained to the families that monitoring was taking place. At the follow-up visit all data were downloaded *via* USB. Adherence data have already been published [14].

Symptom diary

Participants recorded daily symptom scores, ICS use, rescue bronchodilator use and any oral corticosteroid courses in a symptom diary [17] containing four questions for the child and nine questions for the parent for each day of the study (see supplementary material).

Study visits

History of symptom onset, atopic status (see supplementary material for details), gestational age, family history and comorbidities were recorded at the baseline visit. At the baseline and follow-up visit current asthma control was assessed according to GINA guidelines [13], and the asthma control test (ACT) [18] or the childhood ACT (C-ACT) [19] were performed as appropriate. Exhaled nitric oxide (F_{eNO}) was measured using the NIOX VERO (Aerocrine, Sweden) in accordance with American Thoracic Society guidelines [20] (see supplementary material).

Spirometry was performed and forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio, forced expiratory flow at 25–75% of FVC ($FEF_{25–75\%}$) were recorded at the beginning of each visit (Vitalograph Ltd., Buckingham, UK). For lung function assessment, Rosenthal values [21] were used for percent predicted. Z scores were calculated according to Global Lung Initiative 2012 [22]. Spirometry was followed by acute bronchodilator reversibility (BDR) testing. BDR testing was performed 15 min after administration of 1000 µg salbutamol *via* a spacer. Quality of life and psychological comorbidity was assessed using the Paediatric Asthma Quality of Life Questionnaire (PAQLQ) [23]. Current medications were recorded.

For exacerbation assessment, moderate and severe exacerbations in the 3 months prior to each visit were recorded. Severe exacerbations were defined as 1 or more days of oral prednisone prescription. Moderate exacerbations were not severe enough to warrant systemic corticosteroid use and/or hospitalisation (see supplementary material).

Statistical and computational analysis

Data

Measured values of PEF and FEV_1 were included if they fulfilled the technical quality criteria. Measured values of PEF were standardized by calculating z scores using published reference data [21, 24]. Extreme outliers (*i.e.* measurements of a magnitude of 10 median absolute deviations or more from the overall median) were excluded from the analysis. We were not able to perform a power calculation prior to the study, because of insufficient published data.

Computational and statistical methods

The FBC method consists of the following steps:

1. Quantification of similarity in lung function fluctuation between individuals.
2. Grouping of individuals into clusters, such that similarity between members of the same clusters is strong and between different clusters is weak.

Furthermore, the FBC method includes a data-driven process for determining the tolerable amount of missing measurements (see supplementary material). This data-driven process has been described in detail elsewhere [9]. Briefly, a highly compliant subset of patients (*i.e.* with a relatively high number of PEF measurements compared to the expected number of measurements based on the study design), the so-called "gold standard", was selected. More specifically, the gold standard was defined as the subset of those cohort patients whose individual set of PEF measurements contained at least as many measurements as the 60th percentile of the overall distribution of the number of PEF measurements from the whole analysis population. Then, within the gold standard, in order to quantify the similarity in lung function fluctuation between individuals, the distribution of standardized PEF values of a given patient was compared to the distributions of all other patients in the gold standard. This pair-wise comparison was done using the Earth Mover's Distance. Intuitively speaking, the Earth Mover's Distance contemplates the pair of distributions to be compared as piles of sand and measures the effort that it would take to shovel one distribution into the shape and position of the other. These comparisons yielded a collection or "array" of distance values for each participant in the cohort. This collection of distance values constitutes what we call the "lung-function profile" of each patient within the given group of patients that are being compared to each other. The patients within the gold standard were then grouped into clusters based on the similarity of their lung-function profiles. After applying Ward's minimum-variance hierarchical clustering method we obtained clusters, such that similarity between members of the same clusters was strong and was weak between different clusters.

Afterwards, a cluster stability analysis upon random data removal was performed. The outcome of this stability analysis enabled us to establish the minimum number of PEF measurements required to ensure

stability of the clusters identified within the gold standard. Finally, patients who performed this minimum number of PEF measurements were added to the gold standard, and the cluster analysis was repeated with this larger subset to obtain the final clusters.

Once the clusters were identified, clinical characteristics of the cluster members were compared. Distributions of categorical variables were compared among the clusters using Fisher's exact test, whereas distributions of continuous variables were compared using the Kruskal–Wallis test.

Statistical analyses were carried out using the statistical software R [25] or GraphPad Prism. Data were tested for normality using visual inspection, histograms and Kolmogorov–Smirnov testing. Parametric tests were used if the data were normally distributed. If the data were not normally distributed, nonparametric tests were used, or data were logarithmically transformed. The significance level for all tests was set at <0.05. Differences between visit 1 and visit 2 (for each of the parameters measured) were analysed using the Wilcoxon signed-rank test or the paired t-test.

Cluster assignment and classification of patients with incomplete data

The FBC algorithm contains a data-driven mechanism that indicates, based on the relative completeness of each patient's time series of peak flow, which patients to exclude from the clustering procedure, because, if included, the resulting clusters would be unstable and thus unreliable. This mechanism allows us to determine the tolerable levels of missing values that a cohort participant may have in order to be included in the clustering procedure. Given that the compositions of the clusters is not known but needs to be found in a data-driven manner, the requirements on the completeness of each patient's dataset may be relatively high (at least 67 PEF measurements per patient after applying the algorithm to our data).

In a slightly different vein, and not for the sake of testing the stability of the clusters (which is already done within the framework of the FBC method), we explored how the stable clusters described above (determined using relatively complete high-quality datasets) can be used to classify patients with relatively incomplete time series. We thus investigated the potential applicability of the FBC methodology to a clinical scenario where adherence to PEF measurements is notoriously poor. The aim was to use stable clusters obtained from relatively complete cohort data as a database against which new patients are compared. This is done by calculating the distance (see supplementary material for more details) between a new patient's distribution of lung function measurements and the distribution of each member in a given cluster.

The average distance to all the members of a given cluster then defines the distance between the patient and the cluster under consideration. Finally, the patient is *a posteriori* assigned to the nearest cluster by distance, see supplementary figure E1.

Results

Participants

Overall, 120 patients with asthma were recruited (figure 1), consisting of 86 children with difficult-to-control asthma and 34 children with mild-to-moderate asthma. The latter were enrolled as a

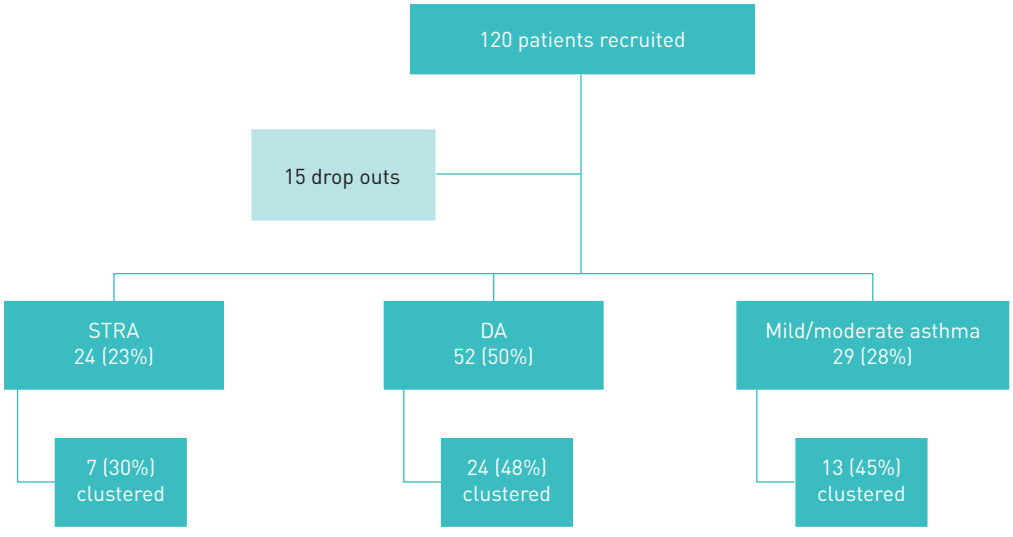

FIGURE 1 Patient population. STRA: severe therapy-resistant asthma; DA: difficult asthma.

control group. Overall, 64 of the children with DA had previously been assessed as part of the difficult asthma protocol and classified as having DA or STRA (a summary of the DA protocol is in supplementary Appendix 1 [11]. A total of 22 patients were newly enrolled in the protocol during the study period. Two of those had been classified in hindsight as STRA (see supplementary Appendix 1 [12]). Overall, 15 patients dropped out of the study (see supplementary Appendix 1).

The baseline characteristics of enrolled patients are shown in table 1. Five patients dropped out because of time constraints, one had focal epilepsy and did not want to participate after a seizure. Four did not attend appointments repeatedly and five lost their device.

There was no significant difference between patients with STRA, DA and mild asthma regarding exacerbations, BDR, F_{eNO}, Mini-Pediatric Asthma Quality of Life Questionnaire (mPAQLQ) and adherence at the follow-up visit (data not shown). FEV_1 only showed a significant difference between those with STRA and mild asthma (p=0.0089). Overall, median adherence was 74% (21–99%).

PEF measurements were recorded during 2–6 months with a median number of 40 (1–121) morning and 26.5 (2–90) evening measurements. Median duration of follow up was 92 days (56–200). Data are incomplete because morning and evening values were not available in all patients. Lung function characteristics of the whole cohort are shown in table 1.

TABLE 1 Demographics of the enrolled population

	Protocol population
Subjects n	105
Male sex	70 (67)
Age years	12.4 (5.4–17.3)
STRA	24 (23)
DA	52 (50)
Mild/moderate asthma	29 (28)
Weight kg	45 (17–101)
Weight centiles	62 (1–100)
Height cm	149 (108–184)
Height centiles	37 (0–99)
Gestational age:	
Term born (>37 weeks)	91 (87)
Preterm (30–37weeks)	13 (12)
Severe preterm (<30 weeks)	1 (1)
Comorbidity	19 (18)
Gastro-oesophageal reflux	23 (22)
Median age of symptom onset months	12 (1–132)
Atopy	92 (88)
Family history of atopy	88 (84)
Parental smoking	19 (18)
Hospitalisation due to asthma in the last year	38 (36)
Treatment	
Rescue medication	105 (100)
Leukotriene-receptor-antagonist	61 (58)
Long-acting β-agonist	100 (95)
Theophylline	10 (10)
Maintenance OCS	8 (8)
Omalizumab	4 (4)
Immunosuppressants (methotrexate)	2 (2)
Median ICS dose µg BDP per day	800 (0–3200)
Median F_{eNO} value ppb	35 (5–196)
FVC % pred	95.3±16.37
FEV_1 % pred	86.1±18.73
Median $FEF_{25–75\%}$ % pred	61 (16–189)
Median BDR %	8.4 (0–123)

Data are presented as n (%), median (range) or mean±SD. STRA: severe therapy-resistant asthma; DA: difficult asthma; BMI: body mass index; ICS: inhaled corticosteroids; BDP: budesonide propionate; OCS: oral corticosteroids; F_{eNO}: fractional exhaled nitric oxide; FVC: forced vital capacity; % pred: % predicted; $FEF_{25–75\%}$: forced expiratory flow at 25–75% of FVC; FEV_1: forced expiratory volume in 1 s; BDR: bronchodilator reversibility in %.

Cluster analysis

We constructed a dendrogram using FBC (figure 2). For PEF data three different clusters were identified. The PEF clusters were, according to the FBC method, stable, and thus reliable, with a minimum number of 67 PEF measurements per patient.

Demographic and lung function characteristics of the different PEF clusters are shown in table 2. The clusters did not differ significantly by age, sex or race but differed significantly in their lung function measurements. The clustered and unclustered patients did not differ significantly except for median adherence and F_{eNO} at follow-up (see supplementary table E3).

PEF clusters (table 3, figure 3)

PEF cluster 1

Cluster 1 contained 27% of all clustered subjects. This cluster had the lowest normalized mean PEF (figure 3). Spirometry was impaired with a mean FEV_1 of 71% (±18.42), statistically significantly lower than in the other two clusters. F_{eNO} was elevated with a mean of 40.5 ppb at baseline and 34 ppb at follow-up, and significantly higher than in the other two clusters. There were 83% of patients with uncontrolled asthma (ACT score <20 of 25), which was significantly more than in the other two clusters. The proportion of STRA was higher than in the other clusters, although this was not statistically significant. Half of the patients had an adherence of ⩽80%.

PEF cluster 2

This contained 45% of all clustered patients. Patients had a statistically higher ICS dose than in the other clusters. Patients had normal spirometry, and the proportion of DA patients (65%) was the highest, although not statistically significant.

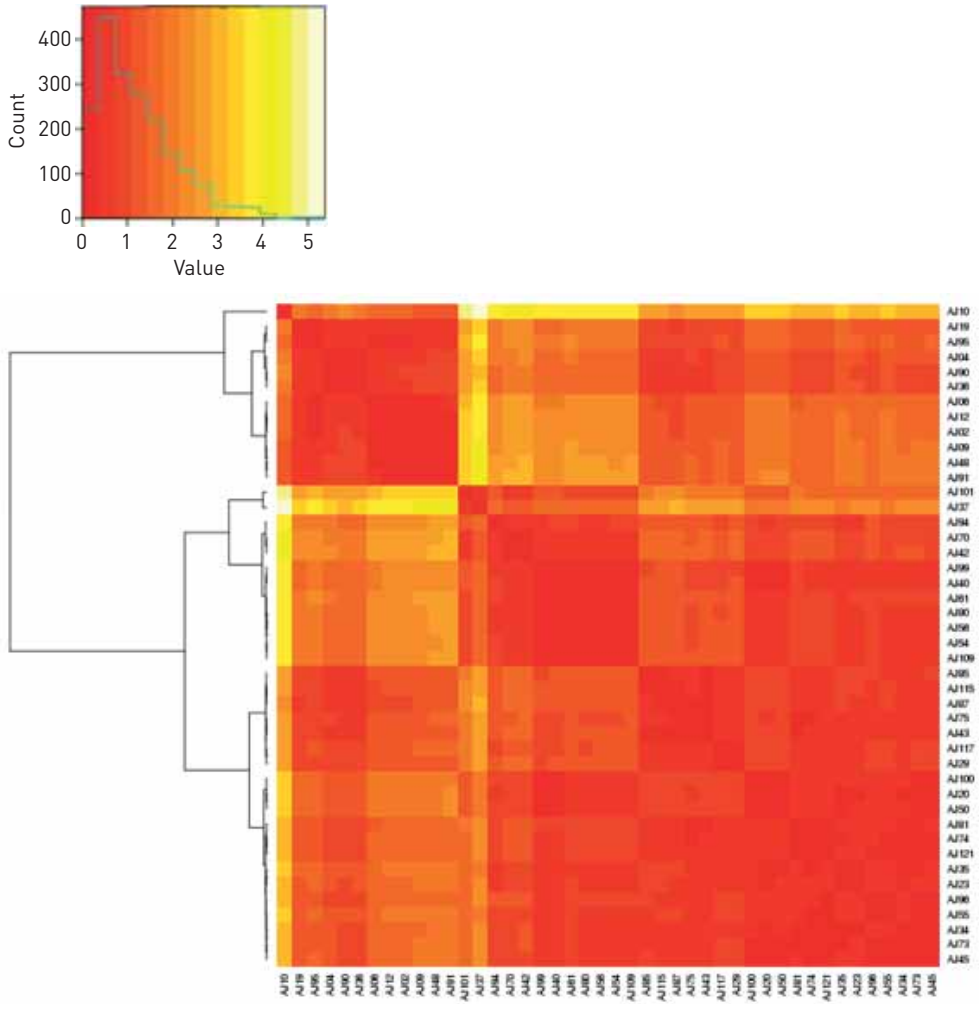

FIGURE 2 Heat map of normalised peak expiratory flow over an entire day. Number of patients included was 44. Minimum number of measurements was 67.

TABLE 2 Comparison of clinical characteristics and different clusters' PEF measurements

	Cluster 1 (n=12)	Cluster 2 (n=20)	Cluster 3 (n=12)	Unclustered (n=61)	p-value
Subjects n	12	20	12	61	
Male sex	8 (67)	11 (55)	8 (67)	43 (70)	0.86
Age years	13.8 (8–17)	12.2 (5–16)	11.8 (7–16)	12.4 (6–17)	0.49
BMI	20 (15–30)	20 (15–32)	19.5 (14–27)	20 (14–39)	0.73
BMI centile	67.5 (9–99)	68 (19–97)	65 (6–98)	72 (0–100)	0.71
Ethnicity: white/other %	67/33	60/40	67/33	48/52	0.46
Comorbidity	5 (42)	2 (10)	2 (17)	10 (16)	0.14
Median age of symptom onset months	15.5 (1–84)	18.0 (1–132)	9.5 (1–72)	12 (1–120)	0.53
Hospitalisations n	0 (0–3)	0 (0–5)	0 (0–5)	0 (0–15)	0.97
Atopic subjects	10 (83)	15 (75)	11 (92)	56 (92)	0.23
LTRA	5 (42)	12 (60)	5 (42)	39 (64)	0.32
Maintenance OCS	2 (17)	1 (5)	1 (8)	4 (7)	0.64
Omalizumab	0	0	1 (8)	3 (5)	0.57
FEV_1 % pred (BL)	71.1± 18.42	94.1± 17.88	92.3± 11.68	85.2±18.56	**0.0057**[#]
FEV_1 % pred (FU)	83.9±15.31	97.3±17.61	102.3±10.23	89.9±15.21	**0.0085**[¶]

Data are presented as n (%), median (range) or mean±SD, unless otherwise stated. BL: baseline; FU: follow-up; BMI: body mass index; LTRA: leukotriene receptor antagonists; ICS: inhaled corticosteroids; OCS: oral corticosteroids; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s. [#]: Bonferroni post-test (Holm–Sidak's multiple comparisons test): significant difference between cluster 1 *versus* 3, cluster 1 *versus* 2, cluster 1 *versus* unclustered; [¶]: Bonferroni post-test (Holm–Sidak's multiple comparisons test) FU: only cluster 1 *versus* 3. Comorbidities were migraine, focal epilepsy and delayed puberty. Bold text indicates statistical significance (p<0.05).

PEF cluster 3

This contained 27% of all clustered patients. This group showed the highest normalised mean PEF (figure 3). Patients had normal spirometry. Comparative analysis showed that control patients with mild-to-moderate asthma were overrepresented in this cluster. This group had 75% of patients with an ACT ⩾20 and 83% with an adherence of ⩾80% (table 3).

The relationship between adherence to medication and measuring lung function on a given day was investigated. To this end, we calculated the four empirical conditional probabilities of taking or not taking medication, conditioned on measuring or not measuring lung function, and *vice versa* (see supplementary material for more details). When medication was taken, it was highly likely that lung function was also measured (median 0.72; interquartile range (IQR) (0.50, 0.87)). If no medication was taken, this probability dropped considerably (median, 0.38; IQR, (0.21, 0.72)) (supplementary table E4). This explains the significant difference seen in adherence in the clustered and unclustered patients (supplementary table E3).

Cluster assignment and classification of patients with incomplete data

In order to explore the feasibility of this procedure and validate the approach, we randomly removed data points from the patients assigned to clusters *via* the FBC methodology, hence generating an "artificial" patient with a "perturbed", incomplete time series of peak flow measurements. These "artificial" patients are proxies for new patients transferred from a clinical setting. We then ran the *a posteriori* cluster assignment procedure on this group of "artificial" patients and checked, for each given "artificial" patient, whether it was *a posteriori* correctly assigned to its original cluster. The results are depicted in figure 4 below as a function of the percentage of data points randomly removed. Up to 80% of PEF data points could be randomly removed and the patients were still correctly *a posteriori* assigned with a classification accuracy of 75% or higher.

Nevertheless, the pattern of missing values is likely to be a property of a patient's characteristics; Thus, random data removal may not capture certain features of those patients with low levels of adherence. Consequently, we explored three possible nonrandom data removal scenarios: The first scenario was data-based and aimed at reproducing the temporal patterns of adherence observed in the entire cohort. The second scenario was based on the hypothetical assumption that a patient, who, on a given day, is not having any symptoms or is having comparatively mild symptoms, will be less likely to conduct a lung function measurement. The third scenario was based on the hypothetical assumption that a patient, who, on a given day, is having comparatively very bad symptoms, will be less likely to conduct a lung function measurement due to an overwhelming sickness feeling. The results of this analysis are depicted in figure 5.

TABLE 3 Clusters: comparative analysis of peak expiratory flow clusters

	Cluster		Cluster 2		Cluster 3		Unclustered		p-value
Subjects n	12		20		12		61		
Asthma severity	**STRA:**	**Mild:**	**STRA:**	**Mild:**	**STRA:**	**Mild:**	**STRA:**	**Mild:**	0.30
	25%	17%	10%	25%	17%	50%	25%	26%	
Median adherence in %	78.6 (60.4–89.1)		85.8 (78.8–87.7)		86.9 (84.2–89.9)		59.5 (42–75.3)		**<0.001**
F_{eNO} ppb (BL)	40.5 (23.5–83.5)		37.5 (11.8–64)		18 (10–26.8)		39 (20–71.8)		0.06
F_{eNO} ppb (FU)	34 (20–61)		13 (9–18)		15 (9–22)		33 (14–60)		**0.002#**
BDR (BL)	**Significant:**		**Significant:**		**Significant:**		**Significant:**		0.35
	50%		20%		33%		30%		
BDR (FU)	**Significant:**		**Significant:**		**Significant:**		**Significant:**		0.45
	17%		10%		0%		16%		
ACT/C-ACT (BL)	**⩾20:**	**<20:**	**⩾20:**	**<20:**	**⩾20:**	**<20:**	**⩾20:**	**<20:**	0.06
	8%	92%	35%	65%	58%	42%	44%	56%	
ACT/C-ACT (FU)	**⩾20:**	**<20:**	**⩾20:**	**<20:**	**⩾20:**	**<20:**	**⩾20:**	**<20:**	**0.02**
	17%	83%	40%	60%	75%	25%	54%	46%	
Median PAQLQ (BL)	**Good:**	**Poor:**	**Good:**	**Poor:**	**Good:**	**Poor:**	**Good:**	**Poor:**	0.97
	36%	9%	47%	5%	50%	8%	53%	5%	
Median PAQLQ (FU)	**Good:**	**Poor:**	**Good:**	**Poor:**	**Good:**	**Poor:**	**Good:**	**Poor:**	0.33
	50%	8%	65%	5%	92%	0%	67%	10%	
Exacerbations in total (BL)	**0:**	**1–3:**	**0:**	**1–3:**	**0:**	**1–3:**	**0:**	**1–3:**	0.57
	50%	50%	30%	70%	25%	75%	38%	62%	
Exacerbations in total (FU)	**0:**	**1–3:**	**0:**	**1–3:**	**0:**	**1–3:**	**0:**	**1–3:**	**0.01**
	42%	58%	75%	25%	83%	17%	43%	57%	
ICS dose	**High:**	**Low:**	**High:**	**Low:**	**High:**	**Low:**	**High:**	**Low:**	**0.01**
	67%	0%	75%	15%	17%	17%	47%	22%	

Data are presented as median (interquartile range), unless otherwise stated. STRA: severe therapy-resistant asthma; F_{eNO}: exhaled nitric oxide fraction; BL: baseline; FU: follow up; BDR: bronchodilator reversibility; ACT: asthma control test; C-ACT: childhood asthma control test; PAQLQ: Paediatric Asthma Quality of Life Questionnaire; ICS: inhaled corticosteroids; mPAQLQ: Mini-Pediatric Asthma Quality of Life Questionnaire; BDP: budesonide propionate. Adherence: good, ⩾80%; moderate, 60–79%; poor, <60%. Exacerbations: 0, 1–3; mPAQLQ: <3, poor; 3–4.9, moderate; 5–7, good; BDR: ⩾12%, significant; <12%, not significant; ICS dose: low, ⩽500 µg BDP; moderate, 500–800 µg BDP; high, >800 µg BDP or equivalent; ACT, C-ACT ⩾20, controlled; <20, uncontrolled; F_{eNO} for children <12 years: <20 ppb, low; 20–35 ppb, moderate; >35 ppb, high; F_{eNO} for children ⩾12 years: <25 ppb, low; 25–50 ppb, moderate; >50 ppb, high. #: Bonferroni post-test (Holm–Sidak's multiple comparisons test): unclustered *versus* clusters 2 and 3. Bold p-values indicate statistical significance (p<0.05).

Discussion

We have shown for the first time in children that clinically relevant asthma phenotypes with different asthma severity can be determined from longitudinal PEF data. Once clusters have been determined, patients can be "*a posteriori* assigned" to the existing clusters. This does not need as many data points as the cluster identification. The *a posteriori* assignment appears to be accurate even if compliance is poor, with up to 80% missing data.

Furthermore, we assessed the clinical relevance of the three clusters found by means of a comparative analysis of clinical characteristics of asthma severity and control parameters. The FBC method was able to detect a functional cluster (cluster 1) of people with asthma with worse lung function, higher inflammatory parameters, worse asthma control, and a tendency to experience more exacerbations. Higher F_{eNO} is a good predictor of high asthma morbidity [26] and worse FEV1 can correlate with the probability of serious asthma exacerbations [27].

The FBC algorithm contains a data-driven mechanism that indicates, based on the relative completeness of each patient's time series of lung function measurements, which patients to exclude from the clustering procedure. If patients with a lot of missing data points were included, the resulting clusters would be unstable and thus unreliable. Therefore, only 42% of all participants could be included in the initial PEF clustering. Interestingly, only 44% of patients were adherent with their asthma inhalers [14]. We found that the main driver of patient behaviour is the need for medication (supplementary table E3). Patients were more likely to measure their PEF on a day when they feel the need to take their medication due to asthma symptoms, which explains why unclustered patients with an insufficient number of PEF values had a significantly lower adherence and F_{eNO} as a possible marker for adherence to inhaled steroids [28] at the follow-up visit. This study has certain limitations and weaknesses. Adherence monitoring had the limitation that only actuation and not the inhalation could be monitored [29]. A limitation of the FBC

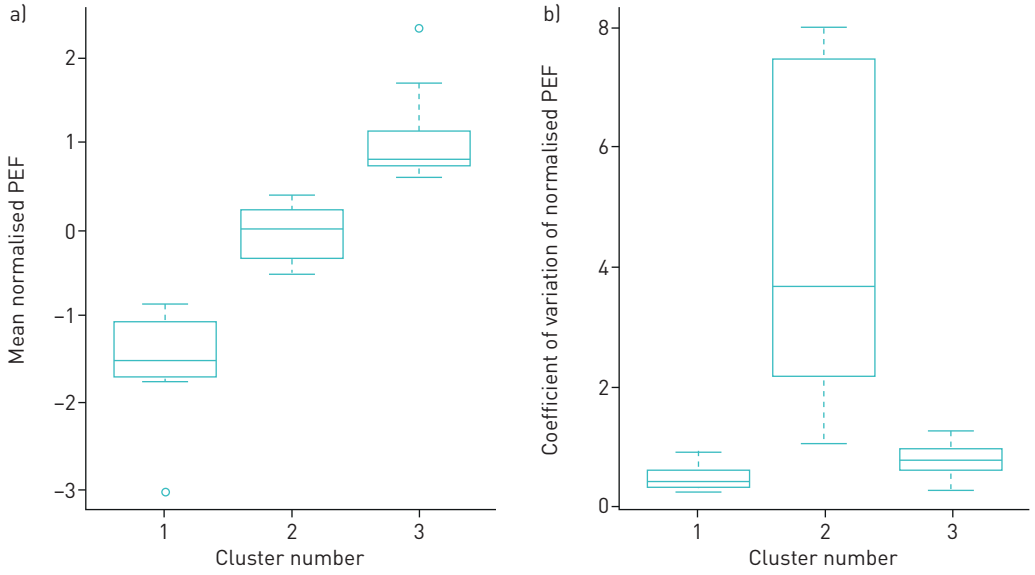

FIGURE 3 Distribution of normalised peak expiratory flow (PEF) within the clusters. a) Empirical distributions of each patient's mean normalized PEF (average over the patient's entire time series of PEF measurements) within each of the clusters. b) Empirical distributions of each patient's coefficient of variation of the normalized PEF within each of the clusters.

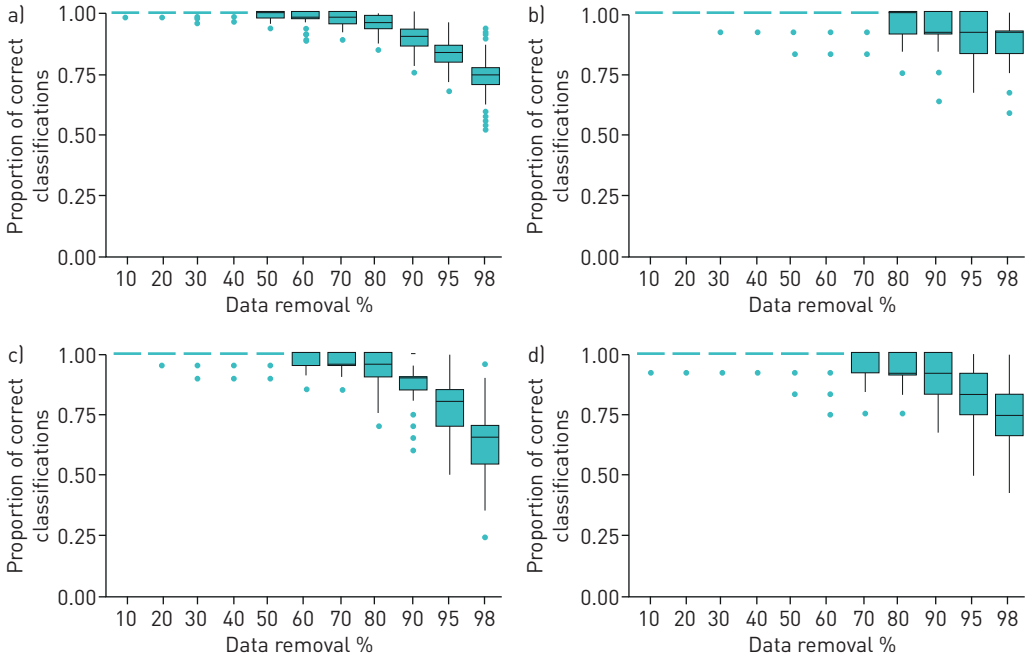

FIGURE 4 *A posteriori* assignment of artificial patients. For a given percentage of data removal (horizontal axis in each panel), in 1000 iterations we randomly removed measurements from the collection of measured PEF values of each patient previously classified *via* fluctuation-based clustering. Thereby, in each iteration, for each previously classified patient, we generated an "artificial" patient lacking a relative amount of data points determined by the value of percentage of data removal at hand. This "artificial" patient was *a posteriori* assigned to a cluster. In each iteration, the proportion of correct *a posteriori* assignments was determined. The results over all 1000 iterations are displayed as a box plot. In the upper left panel, the results are presented for all cluster members. The other three panels show the results only for patients from the cluster specified in the title of the respective panel. a) Overall classification performance, and classification performances for b) cluster 1, c) cluster 2 and d) cluster 3 are shown.

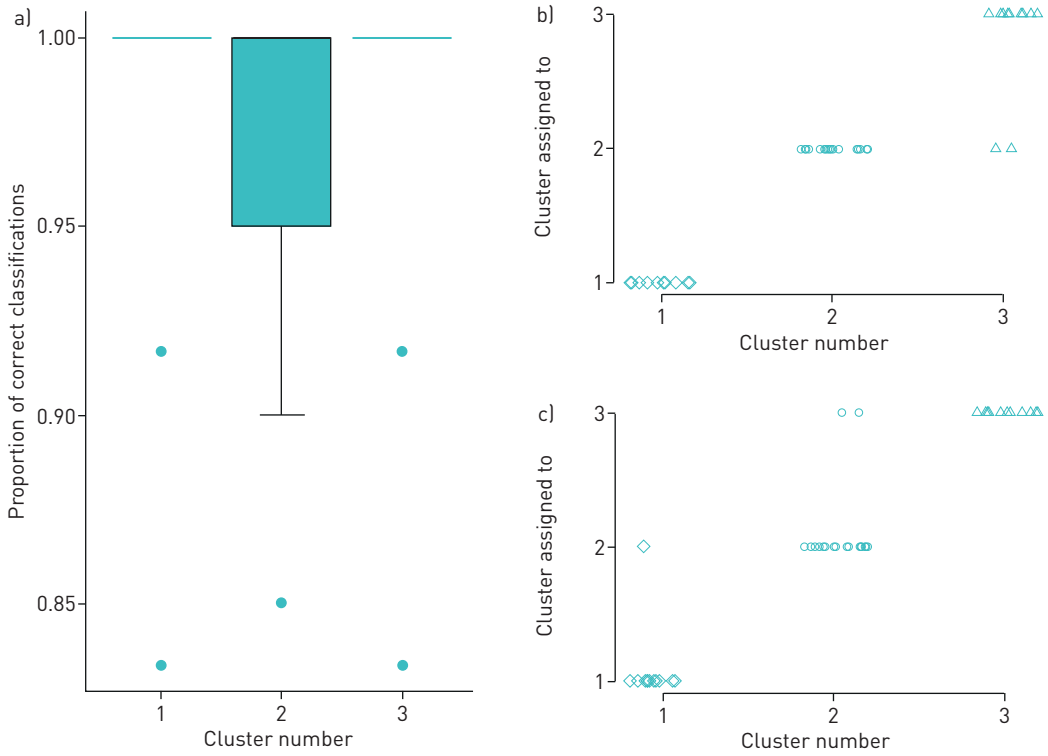

FIGURE 5 Relation between peak expiratory flow (PEF) measurements and adherence. a) Measurements missed on days determined by temporal patterns of adherence in data. In 1000 iterations we randomly removed measurements from the collection of measured PEF values of each patient previously classified *via* fluctuation-based clustering. The data removal was performed according to the temporal patterns of adherence observed in the entire cohort (see Methods for more details). Thereby, in each iteration, for each previously classified patient, we generated an "artificial" patient. This "artificial" patient was *a posteriori* assigned to a cluster. In each iteration, and for each cluster individually, the proportion of correct *a posteriori* assignments was determined. The results over all 1000 iterations are displayed as a box plot. b) Measurements missed on days with zero or mild symptoms. For a given patient classified previously *via* fluctuation-based clustering we systematically removed measurements from the patient's collection of measured PEF values. This was done once for each patient classified previously *via* fluctuation-based clustering. For each patient, data removal consisted of removing all PEF values above the 90th percentile in the patient's collection of PEF measurements. The resulting "artificial" patient was *a posteriori* assigned to a cluster. The results of the *a posteriori* cluster assignments are displayed for members of each cluster separately. c) Measurements missed on days with comparatively bad symptoms. For a given patient classified previously *via* fluctuation-based clustering we systematically removed measurements from the patient's collection of measured PEF values. This was done once for each patient classified previously *via* fluctuation-based clustering. For each patient, data removal consisted of removing all PEF values below the 10th percentile in the patient's collection of PEF measurements. The resulting "artificial" patient was *a posteriori* assigned to a cluster. The results of the *a posteriori* cluster assignments are displayed for the members of each cluster separately.

technique is that patients need to be adherent and competent with doing their PEF measurements otherwise data are unreliable. Data on FEV_1 obtained by these electronic PEF meters are rarely of adequate quality in children due to the short duration of forced expiration. Consequently, this study was focused on PEF.

As FBC is a new technique using a clustering approach for peak-flow measurements to identify different groups of patients with similar clinical characteristics, it is difficult to compare our study with previous work. Adult studies have looked at the fluctuation of peak-flow values to determine asthma stability and found good correlation [8]. Thamrin *et al.* [30] have also shown that PEF fluctuations correlate with the withdrawal of ICS. Other studies have looked at single-point-in-time measurements of lung function, inflammation and asthma control variables [31, 32], whereas this study used longitudinal, twice-daily PEF measurements, which reflect more of the day-to-day reality in asthma [33].

A few questions on interpretation and clinical utility remain unanswered in this study. We do not know the short- and long-term stability of the clusters, nor do we know the extent to which clustering reflects the underlying biology of the disease, or more likely, a combination of endotype, adherence to therapy, and recent exposures to extrinsic triggers such as aeroallergens, respiratory infections, and environmental pollution. This study is a proof of concept, showing that clustering can be performed robustly on relatively limited data. However, we can only speculate as to its clinical utility, for example, determining a group at high risk for an asthma attack who might merit omalizumab therapy [34], or those with true STRA.

This method could prove to be useful as a telemonitoring tool in rural areas, where the next tertiary centre is far away. Further research is needed to determine the accuracy and effectiveness of such an approach.

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