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Dynamics of respiratory symptoms during infancy and associations with wheezing at school age

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ABSTRACT Children with frequent respiratory symptoms in infancy have an increased risk for later wheezing, but the association with symptom dynamics is unknown. We developed an observer-independent method to characterise symptom dynamics and tested their association with subsequent respiratory morbidity.

In this birth-cohort of healthy neonates, we prospectively assessed weekly respiratory symptoms during infancy, resulting in a time series of 52 symptom scores. For each infant, we calculated the transition probability between two consecutive symptom scores. We used these transition probabilities to construct a Markov matrix, which characterised symptom dynamics quantitatively using an entropy parameter. Using this parameter, we determined phenotypes by hierarchical clustering. We then studied the association between phenotypes and wheezing at 6 years.

In 322 children with complete data for symptom scores during infancy (16 864 observations), we identified three dynamic phenotypes. Compared to the low-risk phenotype, the high-risk phenotype, defined by the highest entropy parameter, was associated with an increased risk of wheezing (odds ratio (OR) 3.01, 95% CI 1.15–7.88) at 6 years. In this phenotype, infants were more often male (64%) and had been exposed to environmental tobacco smoke (31%). In addition, more infants had siblings (67%) and attended childcare (38%).

We describe a novel method to objectively characterise dynamics of respiratory symptoms in infancy, which helps identify abnormal clinical susceptibility and recovery patterns of infant airways associated with persistent wheezing.

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Unsupervised analysis of symptom dynamics during infancy identifies subjects susceptible to persistent airway disease http://ow.ly/r9xz30lDpHB

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Introduction

Wheezing disorders in early childhood are a major health issue due to their high prevalence [1] and methods to identify infants at risk for subsequent asthma are therefore needed. Exposure to host factors (*e.g.* sex and maternal atopy) and environmental risk factors (*e.g.* childcare, siblings, environmental tobacco smoke (ETS) and air pollution) influence the incidence and duration of respiratory symptoms during infancy [2, 3], and are associated with wheezing episodes during childhood [4]. In clinical practice, assessing risk factors, estimating the frequency of respiratory symptoms and examining symptom patterns (*e.g.* episodic *versus* persistent symptoms) [5] may help identify infants at risk for later asthma.

The pattern of symptom deterioration and recovery may be especially informative, as it is determined by the symptom dynamics that a subject undergoes during a given period of time. While it is known that persistent wheeze in infants is more closely associated with later asthma and reduced lung function than episodic wheeze [5, 6], no previous study has attempted to characterise symptom dynamics in an observer-independent manner, or to estimate their association with persistent respiratory symptoms at school age.

We hypothesise that symptom dynamics are not only determined by exposure to risk factors related to airway infections (*e.g.* siblings and childcare), but also by host factors and exposure to ETS or air pollution. The symptom dynamics may thus contain information on susceptibility of the airways to infectious triggers. This hypothesis was supported by the previous study of Stern *et al.* [7], in which infants exposed to higher air pollution levels recovered more slowly from viral infections than those with lower exposure levels.

In order to quantify symptom dynamics, we adapted a Markov matrix approach [8]. Previous studies used a Markov model to study the trajectory of asthma severity [9] and asthma control [10]. We took a similar approach by studying the probabilities of transition between different respiratory symptom states using a Markov matrix and further characterised this matrix using the Shannon entropy [11]. Utilising this measure of disorder, we quantitatively characterised the pattern of symptom deterioration and recovery with one single number.

Using this novel method, our aim was to characterise the symptom dynamics of respiratory symptom scores during infancy, assessed weekly for each infant. We tested whether we could identify a symptom dynamic phenotype of infants with higher risk for persistent wheezing and atopic disease (primary outcomes), and allergic sensitisation, lung function measurements and exhaled nitric oxide fraction (F_{ENO}) measurements (secondary outcomes) at school age.

Secondly, in order to facilitate the comparison of this study to previous studies [6, 12, 13], which relied on the total number of infants' symptoms only, we defined similarly sized "reference phenotypes" based on the total number of weeks with respiratory symptoms. We then compared dynamic phenotypes and reference phenotypes relative to the above outcome measures at school age. Lastly, we explored if and how specific dynamic symptom phenotypes are influenced by host factors and environmental risk factors.

Methods

Study design

In the Basel–Bern Infant Lung Development (BILD) birth-cohort study [14], we prospectively assessed weekly respiratory symptom scores (states 0–4) [15] during infancy, resulting in 52 consecutive observations. We used these symptom scores to construct a Markov matrix for each infant. The entries in this matrix are the empirical probabilities of transitioning from any given symptom score to any other within a week's time. These Markov matrices were further characterised using one single quantitative measure, namely the average Shannon entropy.

We first tested whether we could identify symptom dynamics phenotypes based on this entropy parameter using an unsupervised analysis. We defined similarly sized "reference phenotypes" based on the total number of symptom weeks during infancy. Next, we compared associations between symptom dynamics phenotypes and reference phenotypes with wheezing and atopic disease at 6 years (primary outcomes). Allergic sensitisation, lung function measurements and F_{ENO} measurements were secondary outcomes. Lastly, we compared the distribution of risk factors across phenotypes.

Study participants

Unselected, healthy term-born neonates were recruited antenatally in Bern and Basel, Switzerland [14]. Between 1999 and 2015, 369 children from Bern were invited for a follow-up. The Ethics Committees of Bern and Basel approved the study. Written informed consent was obtained from parents before enrolment.

TABLE 1 Respiratory symptom score and associated day and night-time symptoms

Symptom score	Symptoms (cough, wheeze, or breathing difficulty)	
	Day time	Night time
0	None	None
1	Slight; no treatment given	Slight; sleep not disturbed
2	Required treatment but no outside help	Sleep disturbed once; no help required
3	Severe; required help from GP	Sleep disturbed more than once or child needed help
4	Very severe; admitted to hospital	Sleep very disturbed or GP called

GP: general practitioner. Reproduced from [15] with permission from the publisher.

Exposure: respiratory symptoms during infancy

During the first year of life, research nurses called the parents weekly to assess respiratory symptoms using standardised symptom scores with four levels of severity (table 1) [3, 15]. "Weeks with any respiratory symptom" were defined as the total number of weeks a child had a respiratory symptom independent of type or severity, while "weeks with severe respiratory symptoms" were defined as a symptom score of three or more (*e.g.* general practitioner (GP) consultation, as noted previously) [3, 15].

Markov matrices in assessing the dynamics of respiratory symptoms

We used a Markov model approach to examine dynamic behaviour between consecutive symptom scores. We assessed transitions between different levels of symptom scores ranging from healthy (score 0) to most severe (score 4). For each symptom state (*i.e.* initial state, first time point), we counted how often a transition to any other state (*i.e.* target state, second time point) occurred, as assessed during the subsequent week. For each infant, this count information was displayed in a 5×5 matrix (vertical axis=initial state and horizontal axis=target state). These counts were absolute frequencies, which were used to calculate relative frequencies for each transition (figures 1a–c). From this so called Markov matrix [8], we calculated the average Shannon entropy [16] of the empirical conditional probability distributions encoded in each row. Further information on the mathematical approach is provided in figure 2 and the online supplementary material.

Risk factors

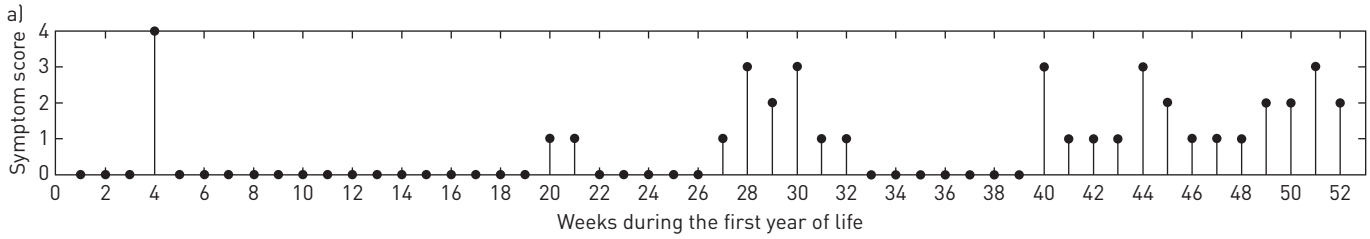
We used a standardised questionnaire to assess pre- and postnatal exposure to risk factors for respiratory symptoms during infancy or asthma development [3]. Parental atopic disease was defined if any of the following was present: self-reported or doctor-diagnosed allergic asthma, history of allergic rhinitis, or atopic dermatitis. Maternal asthma was defined as doctor-diagnosed or self-reported. Maternal education was categorised as low (3 years of secondary education) or high (⩾4 years of secondary education). Duration of breastfeeding (exclusive or nonexclusive) was assessed weekly and binary-coded (<26 weeks or ⩾26 weeks).

Outcomes: respiratory and allergic diseases at 6 years

Asthma and allergy were assessed by an adapted International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. We chose the outcomes "any wheezing" (wheezing between one and 6 years) and "current wheezing" (wheezing over the past 12 months before follow-up). Atopic disease was defined as allergic rhinitis, allergic asthma, or atopic dermatitis. Allergic sensitisation was assessed using a skin-prick test (SPT), determined to be positive if a wheal diameter in any of the tested aeroallergens was greater than in a positive control [14]. Spirometry was performed according to guidelines [17]. Forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio and forced expiratory flow at 25–75% of FVC ($FEF_{25–75\%}$) were expressed as z-scores [18]. We measured online F_{ENO} according to current guidelines [19].

Statistical analysis

Entropy is a measure of disorder within a dynamic system and higher entropy values correspond to more irregularity [11]. The higher the fluctuation between many different respiratory symptoms, the higher is the entropy. For each infant, we calculated the average Shannon entropy [20] over the rows of the Markov matrix, which provided a quantitative measure of the irregularity patterns of symptom deterioration and recovery. Ward's hierarchical clustering [21] was performed to identify phenotypes based on the entropy.



b) Markov matrix written in terms of the absolute numbers of transitions observed

Initial state 0–4	Target state 0–4				
4	1	0	0	0	0
3	0	2	3	0	0
2	0	1	1	2	0
1	2	6	1	2	0
0	26	2	0	1	1
	0	1	2	3	4

c) Markov matrix written in terms of the relative frequencies (in percentage) of transitions observed

Initial state 0–4	Target state 0–4				
4	100	0	0	0	0
3	0	40	60	0	0
2	0	25	25	50	0
1	18.2	54.5	9.1	18.2	0
0	86.7	6.7	0	3.3	3.3
	0	1	2	3	4

FIGURE 1 Distribution of respiratory symptom states during infancy of one infant and corresponding Markov matrices. Panel (a) shows the Temporal pattern of respiratory symptom scores during the first year of life for one infant. We assessed transitions between different levels of the symptom scores (state 0: healthy state, symptom score 0; states 1–4: symptomatic states, symptom scores 1–4). For each symptom state (*i.e.* initial state, 1st time point), we counted how often a transition to any other state (*i.e.* target state, 2nd time point) occurred, as assessed during the subsequent week. This count information is displayed in a 5×5 matrix. Absolute values are displayed in panel (b), whereas the corresponding relative frequencies are displayed in panel (c).

The optimal number of clusters and thereby of phenotypes was determined by the majority of indices [22]. We defined similarly sized "reference phenotypes" by the frequency distribution of the total number of weeks with respiratory symptoms during infancy. Using logistic regression, we studied the association of symptom dynamic phenotypes, reference phenotypes and asthma risk factors with outcomes. For the outcomes any wheezing, current wheezing, atopic disease and positive SPT, analyses were adjusted for sex, maternal education, maternal asthma, ETS exposure, childcare, and siblings. F_{ENO} was additionally adjusted for hay fever and inhaled corticosteroid (ICS) use. For lung function, we adjusted for maternal education, maternal asthma, ETS exposure, childcare and siblings [23]. We used Chi-squared and Kruskal–Wallis tests to compare characteristics across phenotypes and accounted for multiple testing. We used the weighted kappa-statistic to compare agreement between symptom dynamic phenotypes and reference phenotypes.

For sensitivity analyses, we repeated the analysis in infants who had one or more episodes with a symptom score of three or more and also within an additional, independent sample from our cohort of 242 infants. To explore whether the entropy distribution was an artefact of our analysis, we re-categorised the symptom states, simulated data and perturbed the existing data. Furthermore, we assessed the robustness of our findings after correcting for unobserved events. In particular, we theoretically explored the repercussions of observing more severe respiratory symptoms.

Results

From 369 eligible subjects, 322 (87%) were studied (see supplementary figure E1), each having ⩾50 weeks of symptom series during infancy and complete data on risk factors and outcomes (table 2). Demographic data and distribution of respiratory symptoms did not differ between infants followed-up and those lost to follow-up. With respect to risk factors, infants lost to follow-up were more frequently exposed to ETS and were less breastfed (see supplementary table E1).

Distribution of respiratory symptoms

In infants followed-up, we had information for 16 864 person–weeks. The number of weeks with any respiratory symptom was determined (on average) as median 4 weeks (range 0–23 weeks). In contrast, severe symptoms were rare at median 0 weeks (range 0–6 weeks). The distribution of all symptom states is shown in figure 3.

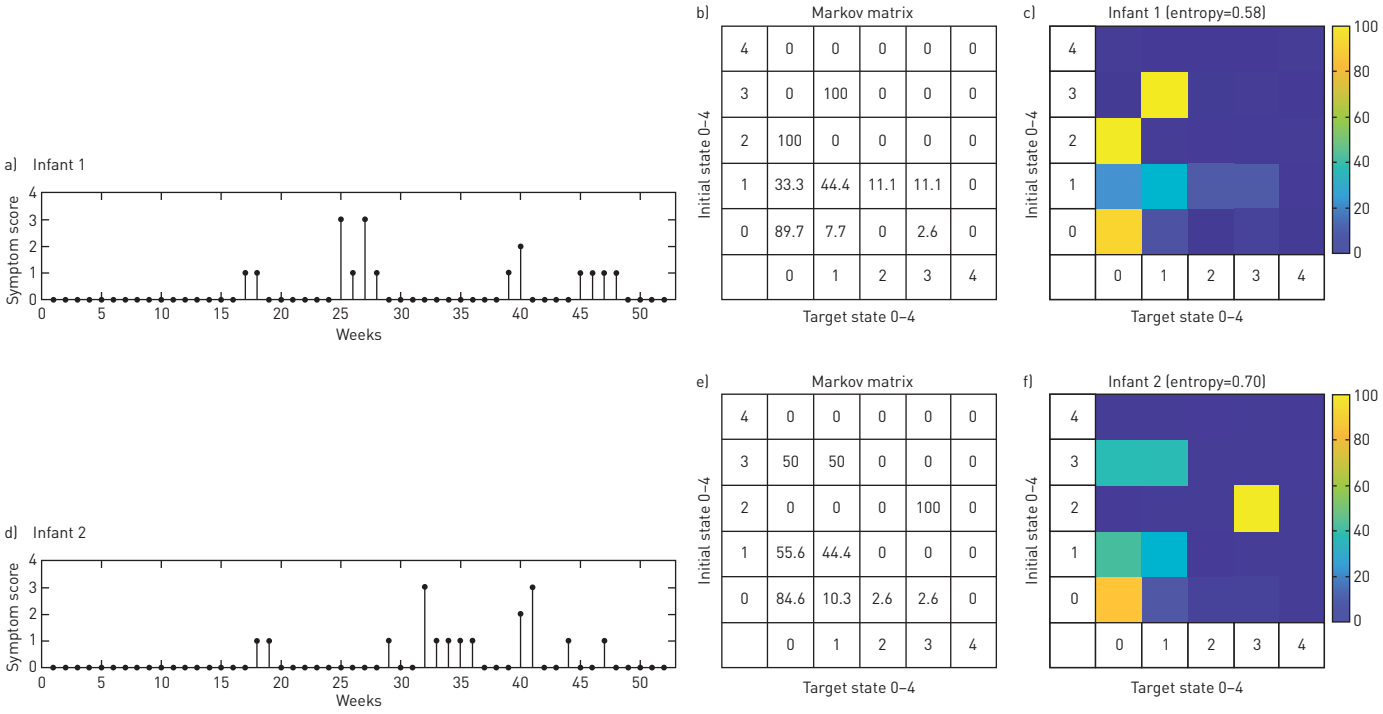


FIGURE 2 Representative Markov matrix patterns of two infants. Panel (a) (infant 1) and panel (d) (infant 2) show the weekly respiratory symptom scores (states 0–4) during the first year of life. The total number of "weeks with respiratory symptoms" during the first year for both infants were the same (37 weeks symptom score 0, 9 weeks symptom score 1, 1 week symptom score 2 and 2 weeks symptom score 3). The Markov matrices in panel (b) (infant 1) and panel (e) (infant 2) show the empirical probability of each transition (described in the methods and figure 1). In panels (c) and (f), the colours correspond to the empirical probability of each transition. The pattern of the Markov matrix landscape was expressed using a single average entropy parameter [16] (described in the supplementary material), which was 0.58 for infant 1 and 0.70 for infant 2.

Dynamics of respiratory symptoms assessed by the Markov matrix

The dynamic patterns of respiratory symptoms for two infants are shown in figure 2. Both had the same number of symptom weeks and the same distribution of symptom scores, but different patterns for the Markov matrix landscape. The shape of the landscape as characterised by a single entropy parameter differed as well. Infant 1 (figures 2a–c) had an entropy of 0.58 *versus* infant 2 (figures 2d–f) who had an entropy of 0.70. The higher entropy value for infant 2 indicates higher fluctuation between respiratory symptom scores compared to infant 1.

Outcomes at 6 years

From 322 children followed-up, 105 (32.9%) had an outcome of any wheezing and 38 (11.7%) had an outcome of current wheezing. There were 120 children (37.5%) with atopic disease and a subgroup of 270 completed a SPT, of which 37 (13.6%) were positive (table 2). Lung function tests were completed in 222 children (68.7%) and 231 (71.7%) had F_{ENO} measurements.

Identification of phenotypes

Hierarchical Ward's clustering was feasible and identified three symptom dynamic phenotypes. We defined three similarly sized reference phenotypes using the frequency distribution of the total number of weeks with respiratory symptoms.

Over- and under-representation of risk factors across phenotypes

Symptom dynamic phenotypes

The three symptom dynamic phenotypes included 145, 135 and 42 infants, respectively (table 3). They differed with regard to the number of weeks with any symptoms and severe symptoms (phenotype 1 had severe symptoms for 0.22 weeks, phenotype 2 for 0.82 weeks and phenotype 3 for 2.11 weeks). Entropy also differed across phenotypes (phenotype 1 had an entropy of 0.08, phenotype 2 of 0.56 and phenotype 3 of 1.03) and so did risk factors. Phenotype 1 had an under-representation of infants with siblings and with childcare attendance, and infants were more likely to be born *via* Caesarean section. Compared to phenotype 1, phenotype 2 had more infants with siblings and with childcare attendance, and infants were less exposed to ETS. In phenotype 3, the smallest group, more infants had siblings and attended childcare,

TABLE 2 Characteristics of the study population

	n (%)	Mean±SD	Median (IQR)	Range
Anthropometric data at birth				
Gestational age weeks		39.6±1.2	39.8 (38.8–40.5)	36.7–41.8
Birth weight kg		3.3±0.4	3.3 (3.0–3.6)	2.1–4.9
Length cm		49.5±1.9	50.0 (48–51)	44–57
Respiratory symptoms in the first year of life				
Weeks with symptoms		5.3±4.6	4 (0–20)	0–23
Weeks with severe symptoms		0.71±1.1	0 (0–1)	0–6
Risk factors				
Male sex	167 (51.8)			
Siblings	159 (49.4)			
Caesarean section	54 (16.7)			
Maternal asthma	34 (10.5)			
Maternal atopy	116 (36.1)			
Childcare	62 (19.5)			
Maternal smoking in pregnancy	27 (8.4)			
Parental smoking during infancy	70 (21.7)			
Breastfeeding >26 weeks	252 (78.3)			
Low maternal education	202 (62.7)			
Season of birth				
Spring	87 (27.4)			
Summer	83 (25.7)			
Autumn	80 (24.8)			
Winter	72 (22.6)			
Outcomes at 6 year follow-up				
Any wheezing[#]	105 (32.9)			
Current wheezing[¶]	38 (11.7)			
Atopic disease[+]	120 (37.5)			
SPT positive[§]	37 (13.6)			

Data are derived from 322 infants with a total of 16 864 observed symptom weeks. IQR: interquartile range; SPT: skin-prick test. [#]: defined as any wheezing episode between one and 6 years of age; [¶]: defined as wheezing over the past 12 months before follow-up; [+]: defined as allergic rhinitis, allergic asthma, or atopic dermatitis before follow-up; [§]: a SPT was completed in a subset of 270 children.

and slightly more infants were male and were exposed to ETS. We considered phenotype 3 a high-risk symptom dynamic phenotype since associations with both later wheezing and atopic disease were strongest.

Reference phenotypes

The three phenotypes included 147, 128 and 47 infants, respectively (table 3). Phenotypes 1, 2 and 3 had 0.14, 0.87 and 2.14 weeks with severe symptoms, respectively. Entropy was 0.13 for phenotype 1, 0.54 for phenotype 2 and 0.91 for phenotype 3. Risk factors differed across phenotypes. Phenotype 1 had an under-representation of males and fewer infants had siblings. Infants were also more likely to be born *via* Caesarean section. In phenotype 2, infants were less likely to be born *via* Caesarean section and in phenotype 3 there was an over-representation of males and more infants had siblings.

Association of symptom dynamic phenotypes, reference phenotypes and risk factors with outcomes at 6 years

In the adjusted logistic regression model, male gender and maternal asthma were significantly associated with any wheezing during childhood. Male gender was also associated with current wheezing, any wheezing and atopic disease. Symptom dynamic phenotype 3 was significantly associated with any wheezing and current wheezing, compared to symptom dynamic phenotype 1, whereas symptom dynamic phenotype 2 was not associated with these outcomes. Reference phenotypes 2 and 3 were significantly associated with any wheezing compared to reference phenotype 1 (table 4). There was a significant association between both maternal asthma and symptom dynamic phenotype 3 toward wheezing between 2 and 3 years of age, whereas reference phenotypes 2 and 3 were not associated with this outcome (see supplementary table E2). None of the phenotypes were associated with the outcome positive SPT (see supplementary table E3). For sensitivity analysis, we repeated the entire analysis and obtained similar

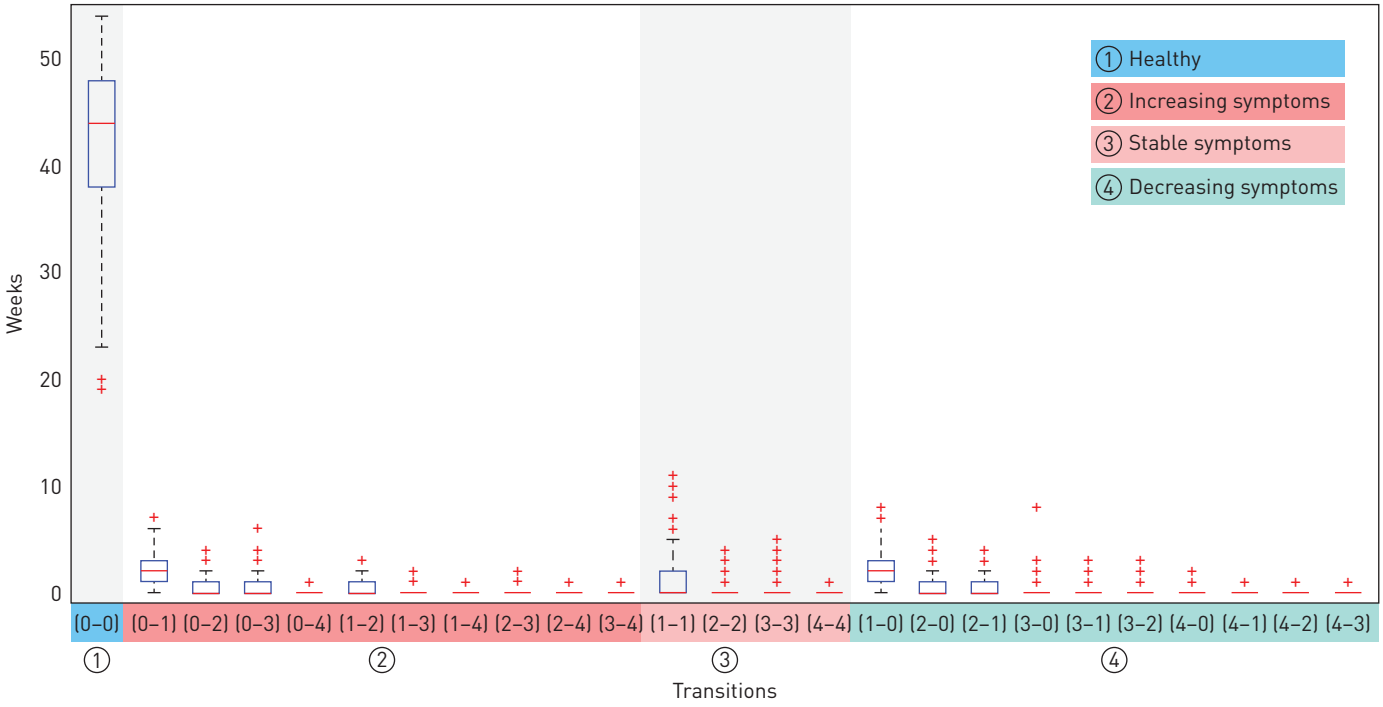


FIGURE 3 Respiratory symptom transitions of all study participants. The vertical-axis represents the number of transitions (in weeks) observed during the first year of life. The horizontal-axis represents all possible transitions (Type 1: healthy state; Type 2: increasing symptoms; Type 3: stable symptoms; Type 4: decreasing symptoms). Data are shown as box plots, while the numbers in brackets indicate initial symptom score (initial state) and target symptom score (target state), as assessed in the subsequent week.

results in infants with one or more episodes with a symptom score of three or more (data not shown). There was no association between any of the predictors and lung function and F_{ENO} measurements at 6 years (see supplementary table E4).

Comparison between symptom dynamic phenotypes and reference phenotypes

There was a high degree of overlap between the dynamic and reference phenotypes, with a weighted kappa-statistic value of 0.61. The overlap of infants allocated to each phenotype was 107 (74%) to phenotype 1, 76 (56%) to phenotype 2 and 20 (48%) to phenotype 3. Infants in dynamic phenotype 3 had greater fluctuation between respiratory symptom scores compared to reference phenotype 3 (entropy 1.03 *versus* 0.91). This indicates that phenotyping based on symptom dynamics identifies infants with different characteristics compared to the reference method based on the number of symptom weeks alone. For example, in high-risk symptom dynamic phenotype 3, we found an over-representation of infants from smoking parents; whereas, in reference phenotype 3, other factors (*e.g.* maternal atopy) were over-represented (table 3).

Sensitivity analyses

The robustness of our findings was assessed in various sensitivity analyses. These are discussed in the supplementary material.

Discussion

We developed a novel method, which, based on a time series of weekly symptom scores from healthy infants in the first year of life, characterised symptom dynamics (*i.e.* the pattern of symptom deterioration and recovery) in an observer-independent fashion. With this method we identified three symptom dynamic phenotypes, of which one had more infants attending childcare and a greater number of infants with siblings. In addition, the number of male infants slightly higher and there was more exposure to ETS. Compared to the reference phenotype, this dynamic phenotype had a slightly higher prevalence for wheeze (26% *versus* 21%) and atopic disease (46% *versus* 36%) at 6 years. Of all clinically measured exposures in infancy in our unselected cohort, infants in this dynamic phenotype had the highest OR for wheeze between one and 6 years (OR 4.31, 95% CI 1.95–9.48) and current wheeze at 6 years (OR 3.01, 95% CI 1.15–7.88). These findings suggest that host factors and susceptibility to environmental factors in

TABLE 3 Characteristics of symptom dynamic phenotypes and reference phenotypes

	Phenotype 1		Phenotype 2		Phenotype 3		p-value	
	Dynamic (n=145)	Reference (n=147)	Dynamic (n=135)	Reference (n=128)	Dynamic (n=42)	Reference (n=47)	Dynamic	Reference
Respiratory symptoms								
Weeks with severe symptoms	0.22±0.43	0.14±0.37	0.82±1.03	0.87±0.98	2.11±1.64	2.14±1.53	**<0.001**	**<0.001**
Weeks with any symptoms	1.84±1.58	1.57±1.09	7.03±3.64	6.61±1.96	12.19±3.56	13.87±3.03	**<0.001**	**<0.001**
Respiratory symptoms transition states								
Entropy of transition states	0.08±0.05	0.13±0.16	0.56±0.14	0.54±0.25	1.03±0.12	0.91±0.23	**<0.001**	**<0.001**
Risk factors								
Male sex	50.3	43.5	49.6	56.3	64.3	65.9	0.223	**0.012**
Siblings	37.2	38.1	57.1	53.9	66.7	72.4	**<0.001**	**<0.001**
Caesarean section	24.8	23.8	10.4	10.2	9.5	12.7	**0.002**	**0.008**
Maternal asthma	9.6	8.8	9.6	10.2	16.7	17.1	0.385	0.278
Maternal atopy	37.2	33.3	34.8	35.9	35.7	44.7	0.914	0.370
Childcare	13.7	11.5	19.3	20.3	38.1	40.4	**0.006**	**<0.001**
Maternal smoking during pregnancy	7.6	6.8	8.2	10.2	11.9	8.5	0.668	0.606
Parental smoking during infancy	24.8	23.1	15.5	20.3	30.9	21.3	0.051	0.805
Breastfeeding ⩽26 weeks	21.4	20.4	21.5	23.4	23.8	21.3	0.941	0.829
Low maternal education	64.1	61.9	62.2	64.1	59.5	61.7	0.851	0.983
Season of birth								
Spring	25.5	26.5	31.1	27.3	19.1	27.7	0.264	0.983
Summer	22.1	18.4	27.4	35.9	33.3	21.3	0.289	0.003
Autumn	35.8	35.4	16.3	15.6	14.3	17.1	**<0.001**	**<0.001**
Winter	16.5	19.7	25.2	21.1	33.3	34.1	0.042	0.111
Outcomes at 6 year follow-up								
Any wheezing[#]	27.6	22.4	28.9	38.3	64.3	51.1	**<0.001**	**<0.001**
Current wheezing[¶]	9.6	8.8	9.6	11.7	26.2	21.3	**0.008**	0.071
Atopic disease[+]	35.4	36.3	37.1	39.4	46.3	36.2	0.449	0.855
SPT positive[§]	13.3	14.5	12.3	13.1	19.4	13.2	0.545	0.942

Data are presented as % or mean±SD. Symptom dynamic phenotypes were defined by the average entropy of transition states and reference phenotypes by weeks with any respiratory symptom. Differences in the distribution of characteristics across phenotypes were assessed using Chi-squared tests for categorical variables and Kruskal–Wallis tests for continuous variables. Significant p-values at the Bonferroni-corrected α-level of 0.017 are shown in bold. SPT: skin-prick test. [#]: defined as any wheezing episode between one and 6 years of age; [¶]: defined as wheezing over the past 12 months before follow-up; [+]: defined as allergic rhinitis, allergic asthma, or atopic dermatitis before follow-up; [§]: a SPT was completed in a subset of 270 children.

TABLE 4 Association of symptom dynamic phenotypes, reference phenotypes and risk factors with outcomes during childhood

Outcome	Univariable association			Multivariable association[#]		
	OR	95% CI	p-value	OR	95% CI	p-value
Any wheezing between one and 6 years[¶] (n=105/322)						
Reference phenotypes						
Phenotype 1 (baseline; n=147)	1	Reference		1	Reference	
Phenotype 2 (n=128)	2.14	1.26–3.62	0.005	1.93	1.11–3.35	0.019
Phenotype 3 (n=47)	3.61	1.81–7.19	<0.001	2.84	1.32–6.11	0.008
Symptom dynamic phenotypes						
Phenotype 1 (baseline; n=145)	1	Reference		1	Reference	
Phenotype 2 (n=135)	1.07	0.63–1.79	0.809	1.06	0.61–1.84	0.830
Phenotype 3 (n=42)	4.72	2.27–9.72	<0.001	4.31	1.95–9.48	<0.001
Risk factors						
Male sex	3.05	1.86–5.01	<0.001	2.90	1.75–4.81	<0.001
Siblings	1.22	0.77–1.95	0.386	1.27	0.78–2.09	0.328
Maternal asthma	2.23	1.09–4.58	0.028	2.16	1.03–4.55	0.041
Childcare	1.49	0.84–2.64	0.169	1.40	0.75–2.59	0.284
Parental smoking during infancy	1.08	0.61–1.89	0.783	0.99	0.55–1.81	0.995
Low maternal education	1.23	0.76–2.01	0.391	1.13	0.66–1.93	0.644
Current wheezing at 6 years[+] (n=38/322)						
Reference phenotypes						
Phenotype 1 (baseline; n=147)	1	Reference		1	Reference	
Phenotype 2 (n=128)	1.36	0.62–2.99	0.433	1.22	0.54–2.75	0.618
Phenotype 3 (n=47)	2.78	1.13–6.86	0.026	2.25	0.83–6.11	0.110
Symptom dynamic phenotypes						
Phenotype 1 (baseline; n=145)	1	Reference		1	Reference	
Phenotype 2 (n=135)	0.99	0.45–2.21	0.994	1.01	0.44–2.31	0.968
Phenotype 3 (n=42)	3.32	1.37–8.01	0.008	3.01	1.15–7.88	0.025
Risk factors						
Male sex	2.52	1.21–5.28	0.014	2.26	1.06–4.81	0.033
Siblings	1.15	0.58–2.28	0.670	1.19	0.61–2.42	0.616
Maternal asthma	1.71	0.65–4.45	0.141	1.66	0.62–4.41	0.306
Childcare	1.35	0.61–3.02	0.462	1.38	0.58–3.27	0.457
Parental smoking during infancy	1.33	0.61–2.89	0.468	1.15	0.51–2.59	0.723
Low maternal education	1.77	0.82–3.78	0.141	1.63	0.73–3.66	0.229
Atopic disease at 6 years[§] (n=120/320)						
Reference phenotypes						
Phenotype 1 (baseline; n=146)	1	Reference		1	Reference	
Phenotype 2 (n=127)	1.13	0.69–1.86	0.602	1.06	0.63–1.78	0.824
Phenotype 3 (n=47)	0.99	0.51–1.97	0.987	0.89	0.41–1.91	0.772
Symptom dynamic phenotypes						
Phenotype 1 (baseline; n=144)	1	Reference		1	Reference	
Phenotype 2 (n=135)	1.07	0.65–1.74	0.778	1.12	0.66–1.88	0.667
Phenotype 3 (n=41)	1.57	0.78–3.17	0.205	1.74	0.81–3.77	0.159
Risk factors						
Male sex	2.15	1.35–3.42	0.001	2.10	1.29–3.40	0.003
Siblings	1.09	0.69–1.72	0.686	1.01	0.63–1.63	0.944
Maternal asthma	1.55	0.76–3.18	0.226	1.51	0.72–3.17	0.274
Childcare	0.62	0.34–1.14	0.127	0.63	0.33–1.21	0.168
Parental smoking during infancy	1.09	0.63–1.88	0.752	0.92	0.55–1.64	0.786
Low maternal education	2.30	1.40–3.77	0.001	2.03	1.20–3.41	0.008

Logistic regression analysis was carried out for the outcomes any wheezing, current wheezing and atopic disease. Symptom dynamic phenotypes were defined by average entropy of transition states and reference phenotypes by weeks with any respiratory symptom. When considering phenotypes as exposure, phenotype 1 from the reference phenotype or symptom dynamic phenotype, respectively, served as baseline. OR: odds ratio. [#]: adjusted for the binary variables male gender, low maternal education, maternal asthma, maternal smoking during pregnancy, childcare attendance during infancy and presence of siblings; [¶]: defined as any wheezing episode between one and 6 years of age; [+]: defined as wheezing over the past 12 months before follow-up; [§]: defined as allergic rhinitis, allergic asthma, or atopic dermatitis before follow-up.

infancy are not only associated with the total number of symptoms, but are associated with symptom dynamics even more so. The same is even truer if we consider the subsequent persistence of airway symptoms into childhood. Although we observed an expected overlap between the symptom dynamic and reference phenotypes, our findings are consistent with the hypothesis that symptom dynamics provide relevant information on airway susceptibility and recovery patterns. The latter may be relevant for our understanding of airway vulnerability in the development of chronic disease.

Comparison with the literature

Previous studies using unsupervised methods assessed symptoms at two timepoints [24] and up to 14 timepoints [12] during childhood. The resolution of respiratory symptoms in our study is unique, since we had a minimum of 50 observations per infant. Comparison of phenotypes between this study and previous studies is limited given that previous studies phenotyped based on "wheeze" during childhood [6, 13, 24–26], while our study focused on "any respiratory symptom" during infancy. Previous studies often investigated the association of phenotypes with risk factors during childhood (*e.g.* allergic sensitisation), while our study specifically assessed prenatal (*e.g.* maternal smoking during pregnancy) and early postnatal risk factors (*e.g.* breastfeeding and childcare attendance). The symptom dynamics and total number of symptom weeks during infancy were not associated with lung function or F_{ENO} at 6 years. These findings differ from the results in a large European birth cohort (the ALSPAC study) describing an association between different wheezing phenotypes (*e.g.* early transient and persistent wheeze) and lung function at 8–9 years [6, 12]. Differences could be due to the small number of lung function measurements performed at 6 years in our study, or that only a few children had severely reduced lung function in this unselected study population. Furthermore, while our method assessed "any respiratory symptom" for phenotyping (mostly cough and rhinitis), the ALSPAC study assessed "wheezing" [6, 12], which may reflect other airway properties.

Potential mechanisms, and interplay between host and environmental risk factors

Interplay between an exposure (*e.g.* virus or pollutants) and host predisposition (*e.g.* infant from asthmatic mothers or male sex) is hypothesised to determine response to a respiratory infection. Studies further suggest that the severity of respiratory infections in children depends on virus type [27], microbial composition [28] and environmental factors [3, 29]. The interplay between these factors is suggested to affect airway epithelial function [30, 31]. Further prospective studies may assess if previously suggested markers to assess epithelial function (*e.g.* cytokine expression and impaired interferon response) [30] are relevant for future disease.

Strengths and limitations

We prospectively assessed respiratory symptoms weekly by telephone interview, obtaining reliable data on respiratory morbidity [15] and reducing potential recall bias compared to retrospectively reported symptoms. We had few missing data points, with only two infants out of all eligible participants being excluded for having less than 50 weeks of observations. We used the same questionnaire to assess risk factors and outcome data throughout the entire study period at both centres, reducing potential inter-centre differences. Since this study was conducted in an unselected population, there were only 38 subjects with current wheezing at 6 years. However, the high-risk phenotypes incorporated only small numbers of infants and as such misclassification of even one subject could majorly influence our findings. While the number of participants lost to follow-up was low (13%), a potential selection bias may still have influenced our findings. Few cohort studies prospectively assessed respiratory symptoms during infancy [3, 13, 32]. Due to a lack of data, we could not validate our method in any of those studies, but did perform an external validation in 242 infants from our cohort not yet seen for follow-up (see supplementary table E5). We performed several sensitivity analyses to rule out that the distribution of entropy was a methodological artefact. For example, we tested whether this distribution could be a mathematical artefact related to the scoring system (five states). However, recategorisation of the states (0, 1, 2, (3+4)→3), did not systematically change the identified phenotypes. After correcting for unobserved events, we investigated the scenario of analysing a hypothetical population at higher risk. This analysis resulted in higher entropy values and also a different entropy distribution (see supplementary material). These results suggest that the phenotypes obtained using our method may depend on the distribution of symptom severity. Consequently, an external validation of our method in a different study population may yield different phenotypes.

Clinical relevance and research applications

Our results indicate that specific symptom dynamics during infancy are associated with wheezing at 6 years. The developed method could potentially be used in ongoing research on asthma control and risk prediction, as it objectively quantifies symptom patterns and could be considered complementary to routine asthma biomarkers (*e.g.* asthma predictive index, F_{ENO} and lung function). Furthermore,

assessment of symptom patterns is noninvasive and does not require laboratory equipment. It could thus be used in primary care and telemonitoring settings [33]. Smartphone applications for the assessment of symptoms are already available and have been used in patients with asthma [34], cystic fibrosis [35], and rhinitis [36]. These novel tools may enable automatic, low-cost assessment of symptoms from which especially high-risk populations may benefit. The mathematical algorithm could be integrated with the tools' software, which would enable the calculation of symptom dynamics without profound expertise, as well as in larger patient groups.

Conclusion

In this study, we developed a method to quantitatively characterise the symptom dynamics of prospectively assessed respiratory symptoms and found that specific dynamic symptom patterns were associated with host and environmental factors and persistent wheezing up to 6 years in a high-risk phenotype. Although host factors (male sex) and environmental factors (ETS exposure during infancy) were more frequent in this phenotype, these infants were predominantly exposed to infectious risk factors (siblings and childcare) in the first year. Beyond solely looking at the total number of symptoms, the dynamics of symptom patterns may provide relevant information on the susceptibility and recovery capacity of the airways after environmental stimuli in infancy. The developed method has potential in various research settings and might contribute to our understanding of airway susceptibility associated with persistent airway disease.

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