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To the Editor

The prevalence of atopic diseases including atopic dermatitis, allergic rhinitis, allergic conjunctivitis, or their combination, and allergic asthma has reached epidemic proportions in Western societies and is still growing.(1) In general; atopic diseases impact quality of life of individuals and contribute to a significant burden for national health care systems.(1)

In this context, many studies have tried to identify biomarkers in umbilical cord blood (UCB) in order to predict development of atopic disease and to identify populations at risk. As such, possible biomarkers for atopic disease may include interleukin 17 (IL17), chemokine (C-C motif) ligand 5 (CCL5), interferon gamma (IFN-γ), and immunoglobulin E (IgE). While IL17 may be indicative especially in severe, uncontrolled asthma,(2) CCL5 was found to be elevated in atopic and asthmatic patients in general.(2) Levels of IFN-γ were inversely correlated with the development of allergy.(3) Also, total IgE (tIgE) levels can be used as a biomarker in this context.(2) Unfortunately, results were so far conflicting and especially evidence in prospective studies is lacking.

Thus, our aim was to assess the association between IL17, CCL5, IFN-γ, and tIgE levels in UCB and the presence of atopic diseases and allergic sensitization at school age. For this, we enrolled unselected, healthy, term born infants from the prospective Basel-Bern Infant Lung Development (BILD) cohort.(4) Midwives collected UCB at birth. After centrifugation for six minutes at 3000 rpm, serum was frozen at -80C for subsequent analyses. Cytokines were measured in duplicates on 96-well plates (50 µl/well) pre-coated with cytokine-specific antibodies using the Human Cytokine Multiplex Assay Kit (IL-17, CCL5, and IFN-γ) according to manufacturer instructions (Bio-Rad, Munich, Germany). Detection limits (pg/ml) of the used assay were 1.55 for IL-17, 1.08 for CCL5, and 1.08 for IFN-γ. For tIgE measurements, semi-quantitative ELISA analyses were

performed at the Institute of Immunology at the University's Hospital of Bern, Switzerland. The following strata were established for tIgE levels: group 1 (<0.5 IU/ml), group 2 (0.51-1.00 IU/ml), group 3 (1.01-5.00 IU/ml), and group 4 (5.01-15.00 IU/ml).

With regard to outcomes of interest and possible confounders, the sequential BILD study design includes several standardized data collections at different moments over time.(4) Parents completed a questionnaire collecting pre- and perinatal data, when the child was 4-6 weeks of age. A follow-up visit at six years of age includes another standardised and validated questionnaire (adapted from the *International Study of Asthma and Allergies in Childhood* – ISAAC)(4) collecting data on atopic diseases and respiratory symptoms. Secondly, a skin prick test against common local allergens (dog dander, cat dander, house dust mite *Dermatophagoides pteronyssinus*, mixed tree pollens, mixed grass pollens, *Alternaria alternata*, a positive control (histamine), and negative control (NaCl 0.9%) (Allergomed, Switzerland) was performed. Tests were determined positive in case of wheal diameters of any tested allergen was equal to or larger than three mm. Based on validated questionnaire data(4), we classified children as atopic in case of parent-reported atopic dermatitis, allergic rhinitis, allergic conjunctivitis (or their combination), allergic asthma, or any combination of these diseases.(5)

We were able to measure UCB tIgE levels in n=143 children and cytokine levels in n=163 children. As some cytokine levels were below detection limits (IL17: n=72/163, CCL5: n=1/163, IFN-γ: n=73/163), this was handled with different approaches. In order to address distribution and censoring issues inherent in cytokine data, we used multiple imputation to generate random values for cytokine results under the detection limit, using normal, half-normal and uniform distributions, as well as assigning a fixed value or leaving non-detectable data as missing. In the final model, we included the half-normal distributed results, as the values of this approach were closest to the mean of all approaches, resulting in a normal

distribution. However, the other approaches mentioned above resulted in comparable results (results not shown). For descriptive statistics and regression analyses, STATA version 14.0 (STATA Corporation, College Station, USA) was used. In order to assess whether UCB biomarker levels could predict allergic sensitization or atopic disease at school age, we used logistic regression adjusted for known and potential confounders, in our case sex and parental atopy. Further possible confounders such as caesarean section, antibiotics during pregnancy, breast-feeding, and parental smoking were not significantly associated with outcomes in our models and were therefore not included in the final model. The Ethics Committee of the Canton Bern and the Research Ethics Committee of University Hospital Bern, Switzerland (Inselspital) approved the study. All parents provided written informed consent for this study.

In total, n=611 children were enrolled in our study from birth on (Table 1). Of these, n=416 have already reached follow-up and completed visits at age six; for n=326/416 we additionally had data on atopic disease. Among the n=115 children, for whom we had both data on UCB cytokine levels and atopic disease at age six, n=44 (38.3%) had atopic disease and n=21 (18.3%) had positive SPTs. Furthermore, among the n=143, for whom we had both data on tIgE levels and atopic disease at age six, n=54 (37.8%) had atopic disease and n=27 (18.9%) displayed positive SPTs. Included study participants were representative of the general BILD cohort population in terms of demographics, except for gestational age with a statistically significant, but unimportantly small difference, and presence of parental atopy (Table 1), however, the share of children with atopic disease is slightly higher when compared to all children with follow up at age six (Table 1), despite also being generally comparable to the Swiss population. Children with atopic disease were comparable to children without atopic disease except for parental atopy and, naturally, outcome measures (Table 2a).

There were no differences between children with or without atopic disease at age six for suspected cytokine biomarkers at birth (Table 2a). There were also no differences between children with or without allergic sensitization at age six for tIgE levels irrespective of IgE groups (Table 2b), however, we found significant differences between children with or without atopic disease at age six for tIgE groups 1 and 2, but not 3 and 4 (Table 2a/b). This has to be noted with caution however due to the small number of children in this category (Table 2a/b) and of sensitized children in total in our study population. When we pooled children of tIgE groups 2-4 due to small numbers, the noted differences were not significant for atopic sensitization, but for atopic disease at age six (Table 2b). Otherwise, there were no associations between concentrations of IL17 (odds ratio (OR):1.51, 95% confidence interval (95%CI) 0.80-2.87), CCL5 (1.00, 1.00-1.00), or IFN-γ (0.98, 0.87-1.11) and atopic sensitization, similarly between IL17 (1.40, 0.75-2.58), CCL5 (1.00, 1.00-1.00), or IFN-γ (OR: 0.99, CI: 0.92-1.07) and the presence of atopic disease at the age of six (Table 2b).

In conclusion, we could not detect any associations between IL-17, CCL5, or IFN-γ in UCB and atopic sensitization or presence of atopic disease at age six in our population. However, we found a possible significant association of UCB tIgE after birth, which of note is not able to cross the placenta, with subsequent atopic disease at the age of six. As mentioned earlier, this has to be taken with caution due to the small number of children with atopic disease at age six in our study population. One major advantage of our study approach is the prospective birth cohort of healthy individuals. Worth mentioning, there are important limitations to our study. At birth, feasibility allowed for collection of serum from UCB only, but not for cellular stimulations. Thus, possible differences of cell-specific cytokine expression could not be assessed and this may have resulted in too small differences in biomarker levels between the groups, leading to non-significant associations. Another possible reason for our findings is the

relatively small sample size of sensitized children and of children with atopic disease in our study population reflecting the unselected healthy nature of the BILD cohort study participants. However, the subsample was representative for the whole BILD cohort population. Further studies with larger sample sizes and using stimulated blood samples for biomarker measurements are needed to re-evaluate these findings.

(1,333 words)

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Table 1

Demographics	Whole study population at age 4 weeks	Children with follow-up data at age 6	Children with both cytokine and follow-up data at age 6	Children with both tIgE and follow-up data at age 6	P	
Birth						
Number of participants	611	326	115	143	General vs cytokine	General vs IgE
Male (%)	328 (53.68%)	174 (53.37%)	59 (51.30%)	74 (51.75%)	0.57	0.6
Gestational age in weeks (SD)	39.63 (1.32)	39.61 (1.41)	39.94 (1.14)	39.78 (1.27)	**0.007**	0.14
Birth weight in kg (SD)	3.37 (0.45)	3.36 (0.47)	3.43 (0.44)	3.40 (0.43)	0.13	0.42
Birth length in cm (SD)	49.51 (2.07)	49.51 (2.10)	49.70 (1.94)	49.62 (1.92)	0.27	0.47
Parental atopy (%)	344 (56.30%)	188 (57.67%)	74 (64.35%)	90 (62.94%)	***0.059***	***0.075***
Parental smoking (%)	108 (17.68%)	43 (13.19%)	24 (20.87%)	43 (30.07%)	0.35	0.54
Follow up						
Age		6.04 (0.30)	6.07 (0.26)	6.08 (0.24)		
Body weight in kg (SD)		22.11 (3.27)	22.52 (3.27)	22.23 (3.21)		
Body length in m (SD)		1.17 (0.05)	1.17 (0.06)	1.17 (0.05)		
SPT positivity (%)		57 (18.63%)	21 (18.26%)	27 (18.88%)		
Any atopic disease (%)		93 (30.39%)	44 (38.26%)	54 (37.76%)		
Symptoms						
Atopic eczema (%)		27 (8.82%)	13 (11.30%)	14 (9.79%)		
Allergic conjunctivitis only (%)		32 (10.46%)	13 (11.30%)	18 (12.59%)		
Allergic rhinitis only (%)		72 (23.53%)	33 (28.70%)	41 (28.67%)		
Allergic rhinoconjunctivitis (%)		37 (12.09%)	17 (14.78%)	23 (16.08%)		
Asthma (%)		10 (3.27%)	4 (3.48%)	6 (4.20%)		

Demographics of the BILD cohort compared to the sub-population with follow-up at age six. If not mentioned otherwise, means and their standard deviation are displayed. Data only shown for participants with complete data. For children with cytokine data, there is also complete data on tIgE. SD: Standard Deviation, N: number of participants, tIgE: total immunoglobulin E.

Table 2a

	Atopic disease at age 6	No atopic disease at age 6	p-value
Birth			
Number of participants	96	230	
Sex			
Male (%)	57 (59.37%)	117 (50.87%)	0.16
Gestational age in weeks (SD)	39.73 (1.32)	39.55 (1.45)	0.29
Birth weight in kg (SD)	3.35 (0.48)	3.36 (0.47)	0.74
Birth length in cm (SD)	49.38 (2.12)	49.56 (2.09)	0.47
Parental atopy (%)	67 (69.79%)	121 (52.61%)	**0.0042**
Parental smoking (%)	16 (16.67%)	27 (11.74%)	0.23
Biomarker levels in UCB			
N	44	71	
IL17 in pg/ml (SD)	1.07 (1.03)	0.76 (1.04)	0.13
CCL5 in pg/ml (SD)	231.88 (146.55)	213.87 (105.08)	0.45
IFNG in pg/ml (SD)	2.06 (2.96)	6.40 (37.85)	0.45
N	54	89	
tIgE group 1 (<0.5 IU/ml) (%)	32 (59.26%)	69 (77.53%)	**0.02**
tIgE group 2 (0.51-1.00 IU/ml) (%)	14 (25.93%)	9 (10.11%)	**0.01**
tIgE group 3 (1.01-5.00 IU/ml) (%)	6 (11.11%)	7 (7.87%)	0.51
tIgE group 4 (5.01-15.00 IU/ml) (%)	2 (3.70%)	4 (4.49%)	0.82
Follow up			
Age in years (SD)	6.07 (0.26)	6.07 (0.25)	0.87
Body weight in kg (SD)	22.05 (3.32)	22.13 (3.25)	0.85
Body length in cm (SD)	116.71 (4.89)	117.59 (5.55)	0.21
SPT positivity (%)	24 (25.00%)	28 (12.17%)	**0.0006**
Symptoms			
Atopic eczema (%)	27 (28.13%)	0	
Allergic conjunctivitis only (%)	33 (34.38%)	0	
Allergic rhinitis only (%)	19 (19.79%)	0	
Allergic rhinoconjunctivitis (%)	39 (40.63%)	0	
Asthma (%)	10 (10.42%)	0	

Demographic characteristics of the children with and without atopic disease. If not mentioned otherwise, means and their standard deviation are displayed. For children with cytokine data, there is also complete data on tIgE. SD: Standard Deviation, N: number of participants, IL17: Interleukin 17, CCL5: chemokine (C-C motif) ligand 5, IFN-γ: interferon gamma, tIgE: total immunoglobulin E, SPT: skin prick test, IU: international unit.

	Atopic sensitization at age 6						
		Raw results without imputation		Half-normal distribution		Mean across all approaches	
	n	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
IL-17	140	1.22	0.82-1.81	1.51	0.80-2.87	1.38	0.83-3.90
CCL5	140	1.00	1.00-1.00	1.00	1.00-1.00	1.00	1.00-1.00
IFN-γ	140	0.99	0.89-1.09	0.98	0.87-1.11	0.99	0.89-1.09
	N	**OR**	**95% CI**				
tIgE group 1	101	1	1				
tIgE group 2	23	1.17	0.42-3.28				
tIgE group 3	13	1.25	0.36-4.31				
tIgE group 4	6	4.70	0.83-26.50				
tIgE groups 2-4 pooled	42	1.37	0.61-3.06				
	Any atopic disease at age 6						
		Raw results without imputation		**Half-normal distribution**		**Mean across all approaches**	
	N	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
IL-17	164	1.33	0.92-1.91	1.40	0.75-2.58	1.38	0.84-2.28
CCL5	164	1.00	1.00-1.00	1.00	1.00-1.00	1.00	1.00-1.00
IFN-γ	164	0.99	0.94-1.04	0.99	0.92-1.07	0.99	0.93-1.06
	N	**OR**	**95% CI**				

tIgE group 1	101	1	1
tIgE group 2	23	**3.68**	**1.40-9.64**
tIgE group 3	13	1.80	0.53-6.10
tIgE group 4	6	1.39	0.22-8.80
tIgE groups 2-4 pooled	42	**2.60**	**1.21-5.58**

Table 2b

Odds Ratios (OR) and 95% confidence intervals (CI) for comparisons of biomarkers with allergic sensitization and presence of any atopic disease at the age of 6, displaying the model "half-normal distribution" and all models combined. N: number of participants, IL17: Interleukin 17, CCL5: chemokine (C-C motif) ligand 5, IFN-γ: interferon gamma, tIgE: total immunoglobulin E, SPT: skin prick test.