Unraveling heterogeneity in pediatric atopic dermatitis: Identification of serum biomarker based patient clusters

Daphne S. Bakker, MD,[a,b] Marlies de Graaf, MD, PhD,[a] Stefan Nierkens, PhD,[b] Eveline M. Delemarre, MD, PhD,[b] Edward Knol, PhD,[b] Femke van Wijk, PhD,[b] Marjolein S. de Bruin-Weller, MD, PhD,[a] Julia Drylewicz, PhD,[b]* and Judith L. Thijs, MD, PhD[a]* *Utrecht, The Netherlands*

Background: Increasing evidence shows that pediatric atopic dermatitis (AD) differs from adult AD on a biologic level. Broad biomarker profiling across a wide range of ages of pediatric patients with AD is lacking.
Objective: Our aim was to identify serum biomarker profiles in children with AD aged 0 to 17 years and compare these profiles with those previously found in adults with AD.
Methods: Luminex multiplex immunoassays were used to measure 145 biomarkers in serum from 240 children with AD (aged 0-17 years). Principal components analysis followed by unsupervised k-means clustering were performed to identify patient clusters. Patients were stratified into age groups (0-4 years, 5-11 years, and 12-17 years) to assess association between age and cluster membership.
Results: Children aged 0 to 4 years had the highest levels of T_H1 cell–skewing markers and lowest levels of T_H17 cell–related markers. T_H2 cell–related markers did not differ significantly between age groups. Similar to the pattern in adults, cluster analysis identified 4 distinct pediatric patient clusters (T_H2 cell/retinol–dominant, skin-homing–dominant, T_H1 cell/T_H2 cell/T_H17 cell/IL-1–dominant, and T_H1 cell/IL-1/eosinophil–inferior clusters). Only the T_H1 cell/T_H2 cell/T_H17 cell/IL-1–dominant cluster resembled 1 of the previously identified adult clusters. Although no association with age or age of onset seemed to be found, disease severity was significantly associated with the skin-homing–dominant cluster.
Conclusion: Four distinct patient clusters based on serum biomarker profiles could be identified in a large cohort of pediatric patients with AD, of which 1 was similar to previously identified adult clusters. The identification of endotypes driven by distinct underlying immunopathologic pathways might be useful to define pediatric patients with AD who are at risk of persistent disease and may necessitate different targeted treatment approaches. (J Allergy Clin Immunol 2022;149:125-34.)

Key words: *Atopic dermatitis, pediatric, biomarkers, endotypes, personalized medicine, principal components analysis, cluster analysis*

Atopic dermatitis (AD) is the most common chronic inflammatory skin disease, affecting up to 20% of children and up to 10% of adults.[1-3] AD can present at all ages, but it mostly begins in early childhood. Although the general consensus is that most pediatric patients with AD will eventually "outgrow" the disease, recent studies suggest that persistence into adulthood is more common than previously recognized.[4] The clinical presentation and distribution of AD in childhood and in adulthood are clearly different,[5-7] and atopic comorbidities, including food allergy, asthma, and allergic rhinitis, develop over the course of infancy and childhood, which is described as the "atopic march."[8,9] In addition to the well-known differences in clinical presentation, increasing insights into blood and skin profiles have shown substantial differences between pediatric AD and adult AD.[10-13] Although both populations show significant T_H2 cell activation in skin and blood, early-onset pediatric AD also shows T_H17 cell/T_H22 cell skewing but lacks the T_H1 cell upregulation that is seen in adults.[10,12,13]

In the past decade it has become increasingly clear that on the basis of clinical characteristics, not only is adult AD heterogeneous but different pathophysiologic mechanisms can be defined in different subgroups of patients. In recent studies, we identified 4 clearly differentiated clusters of adult patients with AD , each characterized by a unique serum biomarker profile.[14,15] These clusters might represent endotypes in which the disease is driven by a distinct underlying mechanism. However, heterogeneity on a biologic level has not yet been confirmed in pediatric AD. Although most pediatric biomarker data are based on studies in infants and young children with recent-onset AD, broad blood

From [a]the National Expertise Center for Atopic Dermatitis, Department of Dermatology and Allergology, and [b]the Center for Translational Immunology, University Medical Center Utrecht.
*These authors contributed equally to this work.
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Corresponding author: Judith Thijs, MD, University Medical Centre Utrecht, Department of Dermatology, Heidelberglaan 100, 3584 CX, Utrecht, The Netherlands. E-mail: j.thijs@umcutrecht.nl.

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Abbreviations used
AD: Atopic dermatitis
CCL: C-C Motif chemokine ligand
CTACK: Cutaneous T-cell–attracting chemokine
CXCL: C-X-C motif chemokine ligand
EASI: Eczema Area Severity Index
MCP-1: Monocyte chemoattractant protein-1
MDC: Macrophage-derived chemokine
MMP: Matrix metalloproteinase
PARC: Pulmonary and activation-regulated chemokine
RBP4: Retinol binding protein 4
TARC: Thymus and activation regulated chemokine
TWEAK: Tumor necrosis factor-like weak inducer of apoptosis

profiling in all age ranges of pediatric patients with AD is limited.[10-13]

The increasing understanding of molecular pathways involved in chronic AD has accelerated the development of more targeted systemic therapies for adult and adolescent patients with AD and will eventually move to children.[16] The specific biomarker pathways distinguishing different patient clusters may be particularly meaningful for applying molecularly targeted drugs and defining the most optimal treatment for the individual patient, because different endotypes might respond differently to the particular treatments. In adult AD, few single biomarkers have previously been proposed for the prediction of response to targeted therapies.[17-19] As yet, however, no data have been published for pediatric AD. Besides predicting treatment response, biomarker profiling could also be used for different purposes in children, including early diagnosis of AD, identification of patients at high risk of persistent disease, and prediction of side effects for a given drug.[20] This necessitates even more the identification of pediatric AD endotypes to optimize safe and effective personalized treatment approaches. Early treatment and AD control in pediatric patients may affect the natural history of the disease.

In the present study, we have investigated biomarker profiles in children with AD aged 0 to 17 years and compared these profiles with the previously found adult AD endotypes. We expect that by defining biomarker profiles in pediatric AD, we will eventually be able to predict the course of the disease and optimize personalized medicine approaches.

METHODS

Patients and samples

Serum samples from 240 children aged 0 to 17 years who were diagnosed with AD, as defined by the criteria of Hanifin and Raijka,[21] were retrospectively selected. Sera were collected at the Wilhelmina Children's Hospital (University Medical Center Utrecht) between 2014 and 2017 and stored at –80°C in a biobank until analysis. The exclusion criteria for this study were use of systemic immunosuppressive drugs within 4 weeks before blood sampling. Disease severity was assessed by the Eczema Area and Severity Index (EASI) score. Clinical characteristics were retrospectively extracted from the patients' electronic medical records. Before study inclusion, parents signed institutional review board–approved written consent in accordance with the principles of the Declaration of Helsinki. The protocols of this study were approved by the institutional review board of the University Medical Center Utrecht (Utrecht, The Netherlands).

Serum biomarkers

To characterize disease heterogeneity and identify specific clusters of pediatric patients with AD, we used an in-house validated panel of the analytes listed in Table E1 (available in this article's Online Repository at www.jacionline.org) to quantify the levels of 145 analytes by a multiplex immunoassay based on Luminex technology,[22] as previously described.[15] The panel was selected on the basis of our previous studies in adult AD, with the addition of 2 newly available markers.[14,15] Serum samples that were above or below the assay limits of detection were given values equivalent to the lower limit of quantification divided by 2 or the upper limit of quantification multiplied by 2.

Statistical analyses

Serum biomarker data were stripped of patients with any missing data (1 patient was removed) and subjected to Box-Cox transformation before analyses. To identify differences in serum biomarker levels within different age groups, patients were stratified into 3 age groups: 0 to 4 years, 5 to 11 years, and 12 to 17 years.

The normalized biomarker data were analyzed by using a principal components analysis to reduce the dimensionality of the data and find the optimal number of principal components explaining the majority of variance in the data, followed by k-means cluster analysis to define and visualize clusters in those principal components, as previously described.[14,15] The optimal number of clusters was determined by using the elbow method, which looks at the total within-cluster sum of square as a function of the number of clusters.[23] The optimal number of clusters was selected to be such that adding another cluster would not significantly reduce the total within-cluster sum of square. To investigate the differences between the age groups, we looked *a posterori* to determine whether the defined clusters were associated with the age groups. Additionally, we compared the biomarker profiles found in our cohort of pediatric patients with AD with the previously described biomarker profiles in adult patients with AD.[14,15]

Clinical characteristics and serum biomarker levels between the age groups and patient clusters were compared by using chi-square tests for categoric variables or 1-way ANOVA for continuous variables, followed by pairwise *t* tests or chi-square tests when appropriate. Benjamini-Hochberg correction was used for all multiple comparisons, controlling the false discovery rate. False discovery rate–adjusted *P* values less than .05 were considered statistically significant. The association of serum biomarkers with disease severity was evaluated by using Pearson correlation coefficients. All statistical analyses were performed by using R Project software (version 3.4.1).[24]

RESULTS

Patient characteristics and age groups

A total of 240 pediatric patients with AD (mean age = 8.2 years; SD = 5.5 years) were included. AD disease severity at the moment of sampling ranged from clear to severe, with a mean EASI score of 14.6 (SD = 10.7). Clinical characteristics are summarized in Table I. Disease severity was not significantly different between children aged 0 to 4 years old (n = 77; mean EASI score = 13.3; SD = 10.1), 5 to 11 years (n = 84, mean EASI score = 14.3; SD = 11.3), and 12 to 17 years (n = 79; mean EASI score = 16.2; SD = 10.5) (Table II). The presence of other atopic comorbidities, including asthma ($P = .001$), allergic rhinitis ($P < .001$), and food allergy ($P = .050$), was significantly higher in the oldest age group (chi-square test). The youngest age group (0-4 years) included significantly more males that did the 5- to 11-years and 12- to 17-years age groups (55.8%, vs 31.0% and 39.2%, respectively; $P = .005$; chi-square test).

Serum biomarker levels were first compared between the 3 age groups by using 1-way ANOVA, followed by pairwise *t* tests with Benjamini-Hochberg correction for multiple comparisons (Fig 1 and see Table E2 in this article's Online Repository at www.

TABLE I. Clinical characteristics of the total cohort of pediatric patients with AD

Patient Characteristic	Total group (N = 240)
Age (y), mean (SD)*	8.2 (5.5)
Male, no. (%)	100 (41.7)
Female, no. (%)	140 (58.3)
EASI score (no.), mean (SD)	14.6 (10.7)
Atopic comorbidities, no. (%)	
Allergic asthma	84 (35.0)
Allergic rhinitis	108 (45.0)
Food allergy	87 (36.3)
No atopic comorbidities	77 (32.1)
Age of onset, no. (%)	
0 -1 y	180 (75.0)
2-11 y	48 (20.0)
12-17 y	2 (0.8)
Missing	10 (4.2)

Categoric variables are presented as counts and percentages; continuous variables are presented as means ± SDs.
*Age at the moment of sample collection.

jacionline.org). By applying this supervised approach, the youngest children, aged 0 to 4 years, were characterized by the highest levels of innate, mostly T_H1 cell–skewing markers (IL-18 and MCP1/C-C motif chemokine ligand [CCL2], TNF receptor 2), epithelial proliferation and differentiation (epidermal growth factor), B-cell homing (BLC/C-X-C motif chemokine ligand [CXCL13]), adhesion molecules (P-selectin, and soluble intercellular adhesion molecule), the adipokine adiponectin, and proinflammatory cytokine macrophage migration inhibitory factor. Children aged 0 to 4 years were also characterized by the lowest levels of the T_H17 cell–related marker trappin2/elafin. Children aged 5 to 11 years were distinguished from the other age groups by the highest levels of the TNF superfamily members thymus and activation regulated chemokine [TWEAK]/TNFSF12 and transmembrane activator calcium modulator and cyclophilin ligand interactor/TNFRSF13B. The oldest children (12-17 years old) were characterized by the highest serum levels of markers related to tissue remodeling (matrix metalloproteinase-1 [MMP-1], MMP-3, and MMP-9) and the lowest levels of the adhesion molecule soluble intercellular adhesion molecule and the multifunctional glycoprotein osteopontin. T_H2 cell–related (IL-5, IL-13, thymus and activation regulated chemokine [TARC]/CCL17, macrophage-derived chemokine [MDC]/CCL22, and MCP-4/CCL13) and T_H22 cell–related (IL-22) markers were more highly expressed in children aged 0 to 4 years than in the other age groups, albeit not statistically significantly.

Correlation of biomarkers with disease severity

We next investigated which serum biomarkers were associated with AD disease severity by determining the correlation of each measured serum biomarker with EASI scores in all 240 pediatric patients (Fig 2). Significant positive correlations between disease severity were found for pulmonary and activation-regulated chemokine [PARC]/CCL18 ($r = 0.63$), apelin ($r = 0.53$), IL-1R2 ($r = 0.49$), TARC/CCL17 ($r = 0.48$), MMP-1 ($r = 0.48$), cutaneous T-cell–attracting chemokine (CTACK) ($r = 0.41$), elastase ($r = 0.40$), I309 ($r = 0.40$), MDC ($r = 0.38$), sVCAM ($r = 0.36$), E-selectin ($r = 0.36$), IL-22 ($r = 0.31$), and S100A8 ($r = 0.31$). EASI score was significantly negatively correlated with retinol binding protein-4 (RBP4) ($r = –0.68$), CatS ($r = –0.66$), ACE ($r = –0.48$), IL-25 ($r = –0.36$), IL-26 ($r = –0.36$), NAP2 ($r = –0.35$), and MMP-8 ($r = –0.30$). Overall, the correlation coefficients from these markers with disease severity were comparable between the 3 age groups and the total cohort (see Table E3 in this article's Online Repository at www.jacionline.org).

Characterization of pediatric AD clusters

In the next step, unsupervised analyses were performed on the Box-Cox–transformed serum biomarker data of all 240 pediatric patients with AD to identify distinct patient clusters based on serum biomarker profiles. After principal component analysis, the cumulative percentage of variance showed that the first 50 principal components described at least 90% of the data set's variance (see Fig E2, *A*) and were hence included in the unsupervised k-means cluster analysis (the top 20 markers for the first 3 PCs in Table E4 in this article's Online Repository at www.jacionline.org). As a result of application of the elbow method on the k-means clustering, 4 was indicated as the appropriate number of clusters (Fig 3, *A* and see Fig E2, *B*). Clinical characteristics were compared between the 4 clusters (Table III). The clusters of pediatric patients with AD seemed to not be influenced by age, as age did not significantly differ between the 4 clusters (Table III [$P = .11$, determined by 1-way ANOVA]), and patients from the 3 age groups were equally divided among the 4 clusters (Fig 3, *B* [$P = .074$, determined by the chi-square test]).

Averages of the serum biomarker levels were calculated per cluster and compared by using 1-way ANOVA, followed by pairwise *t* tests with Benjamini-Hochberg correction for multiple comparisons, to characterize the biomarker profiles driving the 4 clusters (Fig 4 and see Table E5 in this article's Online Repository at www.jacionline.org). Cluster 1 was the largest cluster, representing 41% of the pediatric AD population. The patients in cluster 1 had a mean age of 8.7 years (SD =5.7 years) and the lowest mean EASI score (mean = 9.2 years; SD = 5.4). This cluster was distinct from the other 3 in that it had the highest levels of the acute-phase protein retinol binding protein 4 (RBP4). In addition to the levels of the T_H2 cytokines IL-4, IL-5, IL-13, and TSLP, the levels of the T_H17 cell–related cytokines IL-23 and IL-26 were higher in cluster 1 than in clusters 2 and 4 but lower than in cluster 3. Cluster 1 could be defined as the T_H2 cell/retinol–dominant cluster.

Cluster 2 comprised 31% of the patients (mean age = 8.8 years; SD = 5.3 years). The patients in this cluster had a significantly more severe AD than did the patients in the other clusters ($P < .001$), with a mean EASI score of 27.8 (SD = 7.5). This cluster also had the highest incidence of food allergy (53.4%). The biomarker profile of this cluster was characterized by the highest levels of apelin and markers related to skin homing (PARC/CCL18, TARC/CCL17, and CTACK/CCL27), and it had the lowest levels of markers related to tissue remodeling and angiogenesis (adiponectin, MMP-8, and TIMP1). All of these markers were also strongly correlated with EASI score. Cluster 2 was defined as the skin-homing–dominant cluster.

Cluster 3 represented 18% of the patients; they had a mean age of 6.9 years (SD = 5.4 years) and mean EASI score of 10.5 (SD = 9.1). Cluster 3 was uniquely defined by having the highest levels of biomarkers related to the T_H1 cell pathway (IL-2, IL-12, IFN-α, IFN-γ, TNF-α, TNF-ß, MIG/CXCL9, and ITAC/CXCL11), the T_H2 cell pathway (IL-4, IL-5, IL-13, eotaxin-3/CCL26,

TABLE II. Clinical comparison of the 3 age groups of patients with pediatric AD

Clinical characteristic	0-4 y (n = 77)	5-11 y (n = 84)	12-17 y (n = 79)	*P* value
Age (y), mean (SD)*	2.0 (1.4)	7.6 (1.9)	14.9 (1.7)	<.001
Male, no. (%)	43 (55.8)	26 (31.0)	31 (39.2)	.005
Female, no. (%)	34 (44.2)	58 (69.0)	48 (60.8)	
EASI score (no.), mean (SD)	13.3 (10.1)	14.3 (11.3)	16.2 (10.5)	.271
Atopic comorbidities, no. (%)				
Allergic asthma	15 (19.5)	30 (35.7)	39 (49.4)	.001
Allergic rhinitis	15 (19.5)	40 (47.6)	53 (67.1)	<001
Food allergy	21 (27.3)	29 (34.5)	37 (46.8)	.050
No atopic comorbidities	37 (48.1)	27 (32.1)	13 (16.5)	<.001
Age of onset, no. (%)				.084
0-1 y	63 (81.8)	64 (76.2)	53 (67.1)	
2-11 y	11 (14.3)	16 (19.0)	21 (26.6)	
12-17 y	NA	NA	2 (2.5)	
Missing	3 (3.9)	4 (4.8)	3 (3.8)	

Categoric variables are presented as counts and percentages; continuous variables are presented as means with SDs. Clinical characteristics between the age groups were compared by using a 1-way ANOVA or chi-square test when appropriate. *P* values less than .05 were considered statistically significant.
NA, Not applicable.
*Age at the moment of sample collection; distribution of ages among age groups are presented in Fig E1 (available in this article's Online Repository at www.jacionline.org).

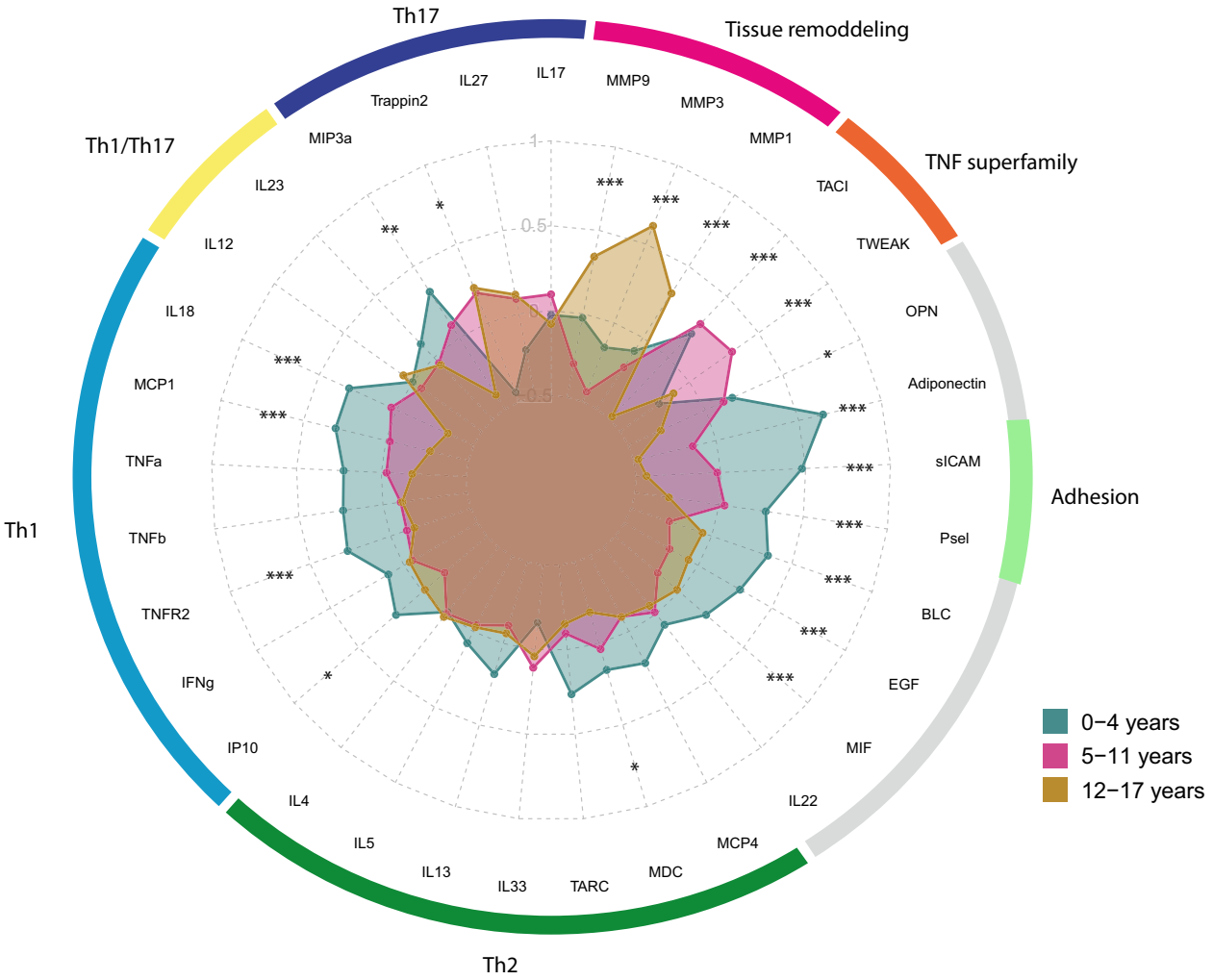

FIG 1. Biomarker profiles in children with AD divided in 3 different age groups. The averages of Box-Cox–transformed serum biomarker levels were compared between children with AD aged 0 to 4 years, 5 to 11 years, and 12 to 17 years at the moment of sampling. Radar plot shows biomarker profiles per age group for selected markers based on significance and AD-related pathways. Spoke lengths represent the means of Box-Cox–transformed data per variable. Significance levels for 1-way ANOVA results are presented with asterisks. *P* values lower than .05 were considered statistically significant. **P* < .05; ***P* < .01; and ****P* < .001.

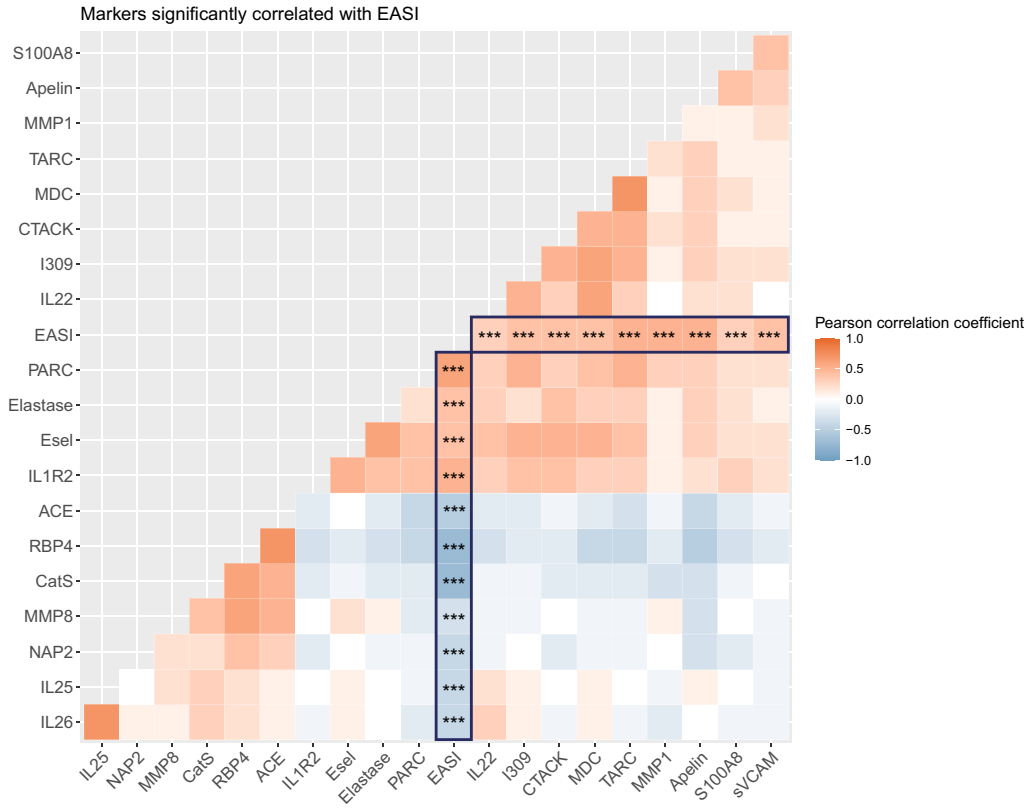

FIG 2. Correlation of disease severity with serum biomarkers in pediatric patients with AD. Heatmap of Pearson correlations between serum biomarkers and AD disease severity measured by EASI score. Heatmap includes only serum biomarkers that are significantly correlated with EASI score and have Pearson correlation coefficients greater than 0.30 or less than or equal to 0.30. Red denotes positive correlations and blue denotes negative correlations. Blue boxes mark correlations between serum biomarkers and EASI score. ***$P < .001$.

TSLP, and MCP-4/CCL13), the T_H17 cell pathway (IL-23, IL-26, MIP3a/CCL20, and GM-CSF), the IL-1 family pathway (IL-1α, IL-1Rα, IL-1R1, IL-18BPa, and IL-37), the TNF superfamily pathway (TNFR1, TNFR2, TWEAK/TNFSF12, and LIGHT/TNFSF14), and T-cell activation (sIL2Rα). Cluster 3 could be described as the T_H1 cell/T_H2 cell/T_H17 cell/IL-1–dominant cluster.

Cluster 4 comprised 10% of the patients; the mean age in this cluster was 6.6 years (SD = 4.9 years), and the mean EASI score was 12.3 (SD = 9.1). The patients from this cluster had the lowest incidence of food allergy (24.0%). Regarding the serum biomarker profile, cluster 4 was distinct from the other 3 clusters in that it had the highest levels of the chemokines RANTES/CCL5 and PF4/CXCL4 and the monocyte activation marker soluble CD14. In addition, cluster 4 showed the lowest levels of biomarkers related to the T_H1 cell pathway (MIG/CXCL9, ITAC/CXCL11, and MIP1b/CCL2), eosinophil trafficking (eotaxin-1/CCL11 and eotaxin-3/CCL26), the IL-1 family pathway (IL1R1 and IL-18BPa), the TNF superfamily pathway (TNFR1, TNFR2, and TWEAK/TNFSF12), neutrophil activation and trafficking (elastase and GCP2), and T-cell activation and skin-homing (sIL2Rα and CTACK). This cluster was defined as the T_H1 cell/IL-1/eosinophil–inferior cluster.

In summary, we were able to identify 4 distinct clusters of patients with pediatric AD. Two of the 4 clusters showed skewing toward the T_H2 cell pathway (clusters 1 and 3), with cluster 3 further characterized by a strong immune activation state related to both innate and T-cell immunity. Cluster 2 was clinically defined by the highest EASI score and was characterized by a biomarker profile skewed toward skin-homing–related markers. In addition to being distinguished by elevation of few innate immunity–related markers, cluster 4 was overall distinguished by a relatively low inflammatory state.

DISCUSSION

This is the first study to broadly characterize serum biomarker profiles in a large cohort of pediatric patients with AD (aged 0-17 years). We confirmed heterogeneity at the level of serum biomarkers in pediatric patients with AD and identified 4 patient clusters based on their unique systemic immune profiles by using an unsupervised clustering approach. Our results suggest unique endotypes in pediatric patients with AD, possibly arguing for personalized, endotype-driven therapeutic approaches rather than the currently used "one-size-fits-all" concept.

The blood biomarker profiles of early-onset pediatric AD have previously been characterized by an upregulation of T_H2 cell, T_H17 cell, and tissue remodeling markers and by a lack of the T_H1 cell upregulation that is seen in adult AD.[10,11,13,25] In contrast to these findings, our pediatric patients with AD aged 0 to 4 years (corresponding in age to the previously studied early pediatric AD cohorts[10-13,26]) was characterized by higher expression of innate

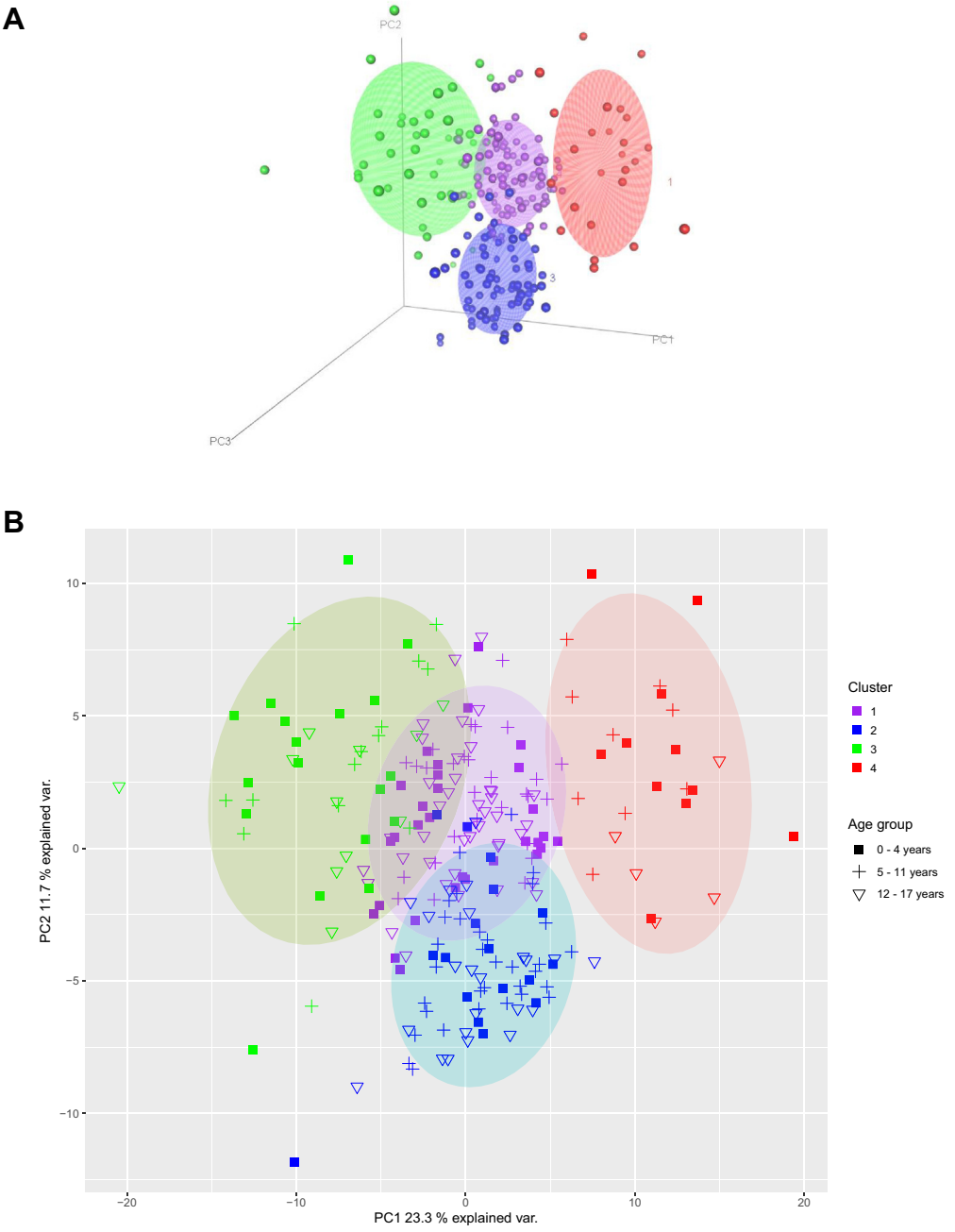

FIG 3. Principal component (PC) and cluster analyses of pediatric patients with AD. **A**, Using unsupervised k-means clustering of the first 50 PCs resulted in the identification of 4 clusters of patients with pediatric AD (clusters 1, 2, 3, and 4). A total of 239 patients are presented in a 3-dimensional plot in terms of the first 3 PCs. Colors and colored ellipses represent clusters. PC1 explained 23.3% of the variance (var.), PC2 explained 11.7%, and PC3 explained 8.6%. **B**, Two-dimensional plots of 239 patients based on the first 3 PCs. Colors and colored ellipses represent clusters. Symbols represent the 3 age groups. Patients from all 3 age groups were equally divided over the 4 clusters.

activation markers that are mostly related to T_H1 cell and decreased levels of the T_H17 cell marker trappin/elafin compared with the older children. The prior studies characterized the blood profiles of pediatric patients with AD within 6 months after disease onset compared with those of the age-matched healthy controls, which might explain the different findings. T_H1 cell–related markers have been identified as markers for disease chronicity and immune development, but in the view of our findings, they might also represent other immune-related mechanisms distinguishing infants and toddlers with AD from older children and adolescents with AD.[27] The innate activation markers were significantly upregulated in the youngest group (IL-18, monocyte chemoattractant protein-1/CCL2, and TNFR2) have been proved to contribute to both T_H1 cell– and T_H2 cytokine–mediated inflammation. In addition, IL-18 and monocyte chemoattractant protein-1/CCL2 are associated with severity of pediatric

TABLE III. Clinical comparison of 4 serum biomarker–based clusters of patients with pediatric AD

Clinical characteristics	Cluster 1 (n = 98)	Cluster 2 (n = 73)	Cluster 3 (n = 43)	Cluster 4 (n = 25)	*P* value
Age (y), mean (SD)*	8.7 (5.7)	8.8 (5.3)	6.9 (5.4)	6.6 (4.9)	.109
Min-Max	0-17	0-17	0-17	0-17	
Male, no. (%)	52 (53.1)	23 (31.5)	20 (46.5)	5 (20.0)	.004
Female, no. (%)	46 (46.9)	50 (68.5)	23 (53.5)	20 (40.0)	
EASI score (no.), mean (SD)	9.2 (5.4)	27.8 (7.5)	10.5 (9.1)	12.3 (9.1)	<.001
Atopic comorbidities, no. (%)					
Allergic asthma	39 (39.8)	27 (37.0)	12 (27.9)	6 (24.0)	.272
Allergic rhinitis	43 (43.9)	38 (52.0)	16 (37.2)	11 (44.0)	.755
Food allergy	29 (29.6)	39 (53.4)	13 (30.2)	6 (24.0)	.017
No atopic comorbidities	33 (33.7)	17 (23.3)	16 (37.2)	10 (40.0)	.267
Age of onset, no. (%)					.384
0-1 y	77 (78.6)	55 (75.3)	26 (60.5)	21 (84.0)	
2-11 y	17 (17.3)	15 (20.5)	13 (30.2)	3 (12.0)	
12-17 y	2 (2.0)	0 (0)	0 (0)	0 (0)	
Missing	2 (2.0)	3 (4.1)	4 (9.3)	1 (4.0)	

Categoric variables are presented as counts and percentages; continuous variables are presented as mean with SDs. Clinical characteristics between the patient clusters were compared by using a 1-way ANOVA or chi-square test when appropriate. *P* values less than .05 are considered statistically significant.
Max, Maximum; *Min*, minimum.
*Age at moment of sample collection.

AD.[28-32] Pediatric AD is supposed to be an even more T_H2 cell–dominant disease than adult AD is. Although not significant, other T_H2 cell–related markers (including IL-5, IL-13, TARC/CCL17, MDC, and MCP-4) were more highly expressed in the youngest children than in the children aged 5 to 17 years. As AD is a primarily T_H2 cell–driven disease, it could be that T_H2 cytokines are upregulated in all pediatric patients with AD and are therefore not different within the 3 age groups. Serum samples from age-matched healthy controls are needed to further investigate this.

The previously described positive correlations of pediatric AD severity with TARC/CCL17, PARC/CCL18, CTACK/CCL27, MDC/CCL22, E-selectin, and the IL-1 decoy receptor IL-1R2 were also present in our study.[10,33-35] MMP-1, an inflammatory marker related to tissue remodeling and previously described to be negatively associated with skin scores in patients with early-onset AD (mean age = 1.8; SD = 1.6 years),[10] showed positive correlation with EASI score in our cohort. In a previous study by Thijs et al,[36] MMP-1 also showed a significant positive correlation with disease severity in adult AD. The difference in the direction of the correlation of MMP-1 with disease severity may therefore reflect age and chronicity of the disease. Retinol binding protein-4 (RBP4) showed a strong negative correlation with EASI scores in our cohort. Both lower retinol levels and RBP4 expression have been detected in skin samples from adult patients with AD, and a negative association of serum retinol with AD severity has been reported in children.[37,38] Retinol has important immunomodulatory effects, and decreased levels of RBP and vitamin A are associated with infection and inflammation.[39-41] These data might support the negative correlation with EASI score in our pediatric cohort as an effect of excessive skin inflammation. In our large pediatric AD cohort, EASI was scored by several different physicians. The subsequent higher interrater variability might have resulted in relatively lower correlation coefficients in our study.

In contrast to previous studies investigating blood and skin biomarkers in pediatric AD,[10-13] our study included a large cohort of children with AD across a wide range of ages and disease durations and it included the use of a quantitative method to measure a broad panel of serum biomarker levels. By using an unsupervised clustering approach, we were able to identify 4 clusters of patients with pediatric AD characterized by specific serum biomarkers that were significantly differentially expressed compared with those expressed in the other clusters. The patients stratified in cluster 1 had the lowest disease severity and were characterized by the highest levels of RBP4, which showed the strongest negative correlation with EASI score. Additionally, the patients in cluster 1 showed higher levels of IL-4, IL-5, IL-13, and TSLP and could be defined as the T_H2 cell/retinol–dominant cluster. Patients in clusters 1 and 3, representing 59% of the patients, shared a T_H2 cytokine–high profile corresponding with the percentage of T_H2 cell–dominant patients, as previously reported in adults.[14,15] These patients would hypothetically be the most ideal candidates for T_H2 cell–targeting drugs.

In contrast to the patients in the other 3 clusters, those in cluster 4 showed a relatively low inflammatory state, with no clear immune skewing, and cluster 4 could therefore be distinguished from the other clusters as being the T_H1 cell/IL-1/eosinophil–inferior cluster. Cluster 4 was defined by elevation of the levels of the monocyte activation markers RANTES/CCL5, PF4/CXCL4, and sCD14. Elevated platelet activation, as shown by higher levels of PF4/CXCL4, has been suggested to play a role in the pathomechanism of chronic skin inflammation in AD, by inducing leukocyte recruitment and through direct activation of local capillary endothelial cells and attraction of effector T-cells to the skin.[42] Both RANTES/CCL5 (a potent eosinophil, monocyte, basophil, and lymphocyte chemoattractant) and the monocyte activation marker sCD14 have shown evidence of association with AD as well.[43-45]

When we focused on the driving pathways in each of the patient clusters, only 1 of the 4 pediatric AD clusters was comparable to 1 of the previously defined endotypes in adult patients with AD.[14,15] The biomarker profile of the pediatric T_H1 cell/T_H2 cell/T_H17 cell/IL-1–dominant cluster 2 was found to correspond to the profile of the T_H1 cell/T_H2 cell/T_H17 cell–dominant cluster identified in adult patients with AD. These results strengthen the previous findings showing that the blood profiles in pediatric AD differ from those of adult patients with AD. However, the identified biomarker-based pediatric patient clusters in the current study

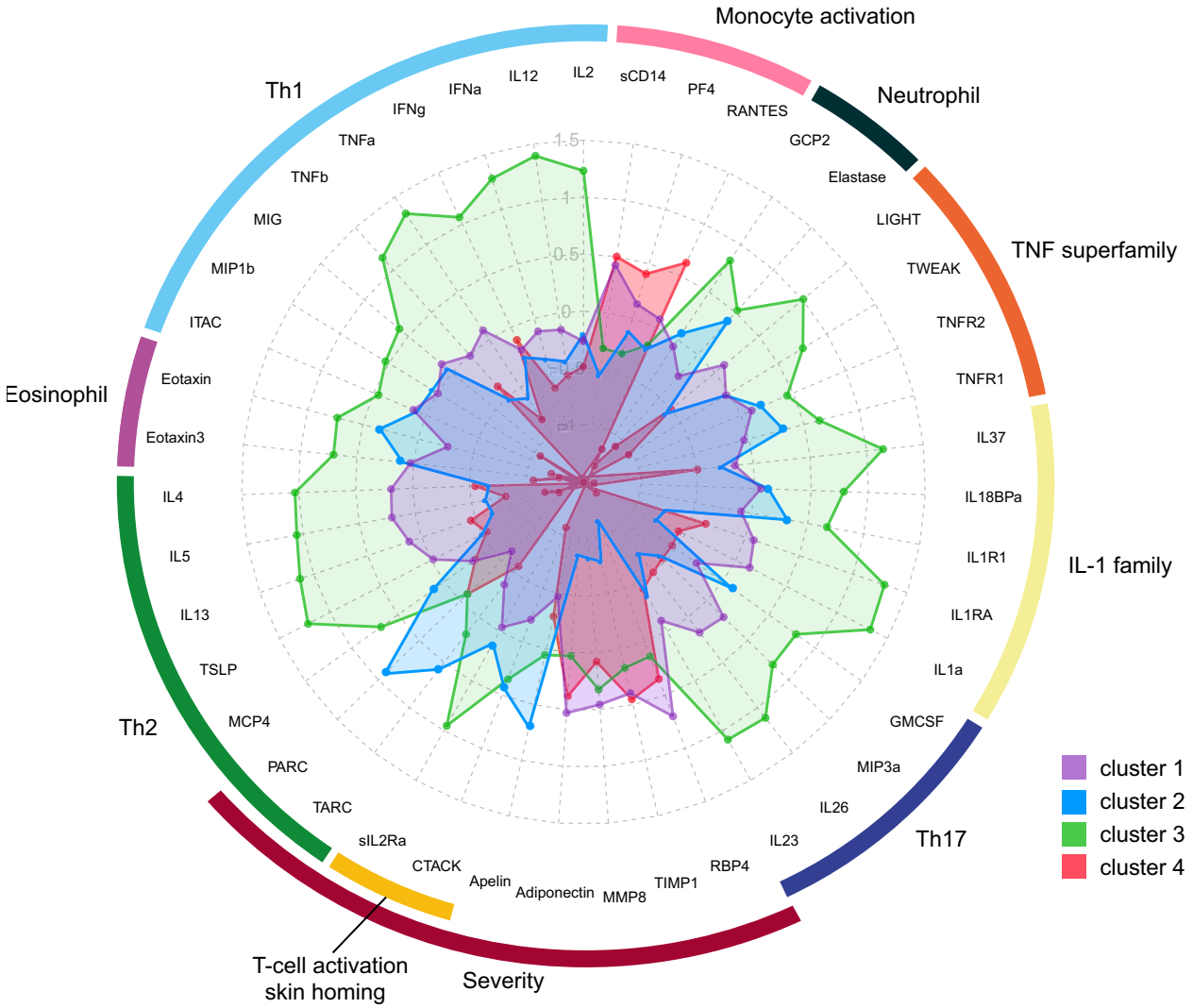

FIG 4. Biomarker profiles of 4 distinct clusters of pediatric patients with AD. Averages of Box-Cox–transformed serum biomarker levels were compared between the 4 identified pediatric AD clusters (clusters 1, 2, 3, and 4). Radar plot shows biomarker profiles per cluster for markers with significantly higher or lower expression in 1 of the clusters versus in the other clusters. Spoke lengths represent means of Box-Cox–transformed data per variable.

were not influenced by age or age of onset. Furthermore, the absolute differences in biomarker levels between the 4 unsupervised identified clusters were more pronounced than the differences that were found by using a supervised approach to compare the 3 age groups. Although 3 of the pediatric patient clusters differ from the previously identified adult AD clusters, our results might indicate that the distinct pathophysiologic mechanisms driving the heterogeneity of pediatric AD cannot be solely assigned to the difference in age or duration of the disease, and they might argue for endotype-specific rather than uniform or age-specific therapeutic strategies.

Among the most important questions regarding disease heterogeneity in pediatric AD are (1) in which patients will the disease resolve and (2) in which patients will it persist into adulthood. One could speculate that patients with resolving childhood AD and patients with persisting disease may represent separate endotypes. Early identification and targeted treatment of the nonresolving endotype might theoretically prevent the atopic march and persistence of AD into adulthood. Previous birth cohort studies have shown that one of the strongest risk factors for nonresolving AD is disease severity and that the presence of asthma and allergic rhinitis did not affect the course of AD.[46-48] In contrast to the previous studies in adults,[14,15] our current study showed that the cluster membership of pediatric patients with AD was influenced by disease severity. Patients in cluster 3 had significantly higher EASI scores than did those in the other 3 clusters. Their driving biomarker profile was characterized by the highest levels of the T_H2 cytokine PARC/CCL18 and apelin and the lowest levels of RBP4, MMP-8, and ACE, all of which are biomarkers that were significantly correlated with EASI scores. The patients in cluster 3 represented 31% of the total cohort, which is consistent with data from studies of large birth cohorts showing that up to one-third of children diagnosed with AD had persistent disease.[46-48] On the other hand, the patients in cluster 2 showed a biomarker profile comparable to that of the adult patients with AD who were previously stratified into the T_H1 cell/T_H2 cell/T_H17

cell–dominant cluster[15] and might thus be considered to have a higher risk of nonresolving AD. Longitudinal follow-up studies are needed to confirm the endotype of each cluster, as well as to investigate whether the persistence of AD is related to 1 of the 4 endotypes and whether endotypes remain stable over time or might change after treatment with systemic immunosuppressive of immunomodulatory drugs. Comparing the profile of cleared versus persistent pediatric AD will better define the biomarker-specific characteristics that predict AD clearance.

Despite inclusion of different age groups, our study was not longitudinal and thus did not follow the same cohort over time. Another limitation is the lack of age- and sex-matched healthy controls, which makes it difficult to distinguish disease-specific from age- and sex-specific differences in biomarker profiles, for instance during puberty, although the patient clusters were not influenced by age. The biomarker panel that was used in this study was based on our previous studies including adult patients with AD[14,15] and was not specifically selected for pediatric patients with AD. The panel was composed of all markers available in our laboratory at the moment of measurement. We believe that the broad panel, which includes many different pathways (eg, T_H1 cell/T_H2 cell/T_H17 cell/T_H22 cell, IL-1 family, cell differentiation, skin-homing, and innate and adapted immunity), covers potential adult- and pediatric-specific markers.

By using an unsupervised profiling approach in our study, we obtained findings indicating that pediatric AD is a biologically heterogeneous disease. We were able to identify 4 distinct patient clusters based on serum biomarker profiles in a large cohort of pediatric patients with AD who were aged 0 to 17 years. Cluster membership was not influenced by age or age of onset, but disease severity seems to be associated with patient clustering. The identification of endotypes driven by distinct underlying immunopathologic pathways might be useful to define pediatric patients with AD who are at risk of persistent disease and may necessitate different targeted treatment approaches. Future longitudinal studies will be needed to further validate the endotypes and may provide additional insights into the stability of the endotypes in pediatric patients with AD over time.

Key messages

- **In a large cohort of patients with AD aged 0 to 17 years, we identified 4 patient clusters based on distinct serum biomarker profiles.**
- **Pediatric AD clusters were influenced by disease severity and not by age or age of onset. Only 1 pediatric AD cluster was comparable to 1 of the previously identified adult AD clusters.**
- **Stratification of pediatric patients with AD into distinct biomarker-based endotypes might help to predict persistent disease and might contribute to more personalized treatment approaches.**

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