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ORIGINAL ARTICLE

Atopic Dermatitis, Urticaria and Skin Disease

Predicting persistence of atopic dermatitis in children using clinical attributes and serum proteins

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Abstract

Background: Atopic dermatitis (AD) is the most common inflammatory skin disease in children, with 30% of all those diagnosed developing chronic or relapsing disease by adolescence. Such disease persistence cannot yet be predicted. The aim of the present study was to predict the natural course of AD using clinical parameters and serum proteins.

Methods: Sera of 144 children with AD (age 0-3 years) were analyzed for IgE and 33 cytokines, chemokines, and growth factors. Patient disease course until the age of 7 years was assessed retrospectively. Unsupervised k-means clustering was performed to define disease endotypes. Identified factors associated with AD persistence at the age of 7 years were validated in children with AD in an independent cohort (LISA Munich; n = 168). Logistic regression and XGBoosting methods followed by cross-validation were applied to predict individual disease outcomes.

Results: Three distinct endotypes were found in infancy, characterized by a unique inflammatory signature. Factors associated with disease persistence were disease score (SCORAD), involvement of the limbs, flexural lesion distribution at the age of 3 years, allergic comorbidities, and disease exacerbation by the trigger factors stress, pollen exposure, and change in weather. Persistence was predicted with a sensitivity of 81.8% and a specificity of 82.4%. Factors with a high impact on the prediction of persistence were SCORAD at the age of 3 years, trigger factors, and low VEGF serum levels.

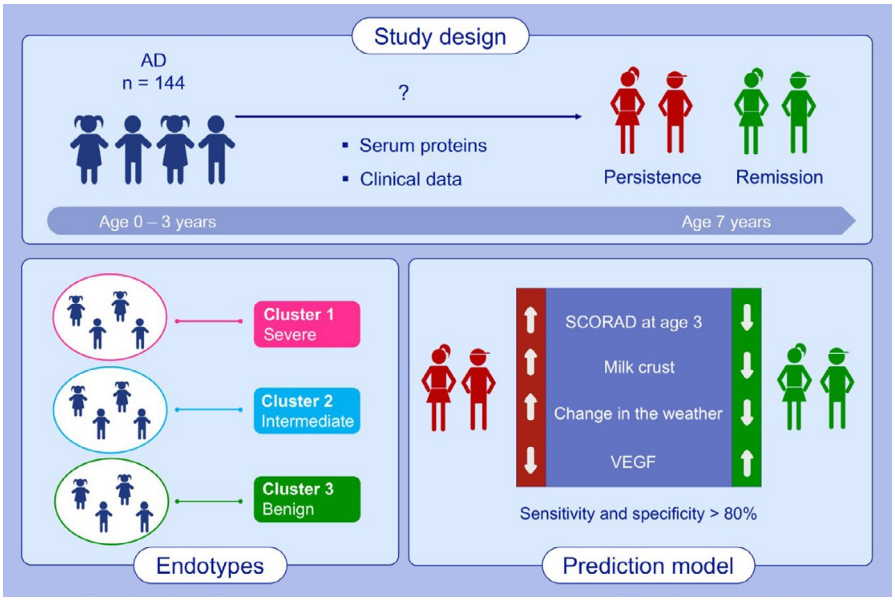
Conclusion: Atopic dermatitis in infancy comprises three immunological endotypes. Disease persistence can be predicted using serum cytokines and clinical variables.

KEYWORDS

atopic dermatitis, atopic eczema, endotype, predictive biomarker

Abbreviations: AD, atopic dermatitis; ARC, allergic rhinoconjunctivitis; IgE, immunoglobulin E; SCORAD, severity scoring of atopic dermatitis.

Felix Lauffer and Veronika Baghin contributed equally



GRAPHICAL ABSTRACT

Three distinct endotypes are found in infant AD. Persistence of AD at the age of 7 years is predicted with a sensitivity and specificity >80%. Factors with a high impact on the prediction of persistence are SCORAD at the age of 3 years, milk crust, aggravation triggered by change in the weather, and low VEGF serum levels.

1 | INTRODUCTION

Atopic dermatitis (AD) is the most common inflammatory skin disease among children, with a prevalence of up to 20%.[1] While the majority of children will undergo disease remission by adolescence, about a third develop chronic or relapsing disease.[2-4] Several risk factors for AD persistence have been proposed, including high disease score at first appearance, family history of atopy, early wheeze, and elevated total serum IgE.[5,6] However, it is still impossible to predict the individual course of AD at an early stage.

A possible reason for this is the heterogeneity of the disease based on a complex interplay between genetics and environmental triggers. An impaired skin barrier caused by, for example, *filaggrin* mutations as well as microbiota and allergens eliciting adaptive and innate type 2 immune responses are the supporting pillars of atopic inflammation.[7-9] In particular, Th2 cells and their key cytokines IL-4 and IL-13 hamper the differentiation and barrier formation of keratinocytes, mediate the release of pro-inflammatory chemokines, and promote the maturation and production of IgE by B cells.[10] Many factors drive the clinical picture of AD in individual patients, thus offering the rationale for the investigation of endotypes and predictive biomarkers. As AD leads to systemic inflammation,[11] serum proteins can be used to identify different immunological endotypes of AD, as well as to objectively score disease severity.[12-16]

In the present study, we hypothesized that not only the clinical phenotypes, but also the heterogeneous course of AD in children rely on different disease endotypes. Early identification of high-risk children would lead to improvements of patient care as these patients and their parents could be offered close medical supervision, adequate drug treatment, and allergy prevention. For this reason, we investigated whether the assessment of serum proteins in combination with clinical variables is useful to stratify children regarding their individual risk of AD persistence. We considered children whose parents observed visible AD skin lesions at the age of 7 years as patients with a persistent AD course.

2 | MATERIALS AND METHODS

2.1 | Study design

2.1.1 | Study and validation cohorts

We identified 248 serum samples of children aged 0-3 years who had received a physician-confirmed diagnosis of AD at the Department of Dermatology between 2005 and 2011. All samples were stored in the Biobank Biederstein at −80°C. We were able to contact 135 patients, of whom 124 were willing to participate in the study. For these 124 patients, we retrospectively gathered clinical information regarding family history and history of disease between the ages of 0 and 3 years (Table S1). To assess individual trigger factors, the parents were asked if they had observed a worsening of AD associated with certain influencing factors, such as vaccination, pollen exposure, or changes in weather (temperature and humidity).

Next, at the age of 7-16 years, the patients and their parents were interviewed personally during an outpatient visit at the department (n = 25), contacted via phone (n = 39), or asked to fill out a questionnaire (n = 60). Two patients in the study cohort were

Asians; the remaining 122 were Caucasians. Persistent AD course of AD was assumed when the parents reported visible skin lesions of AD (SCORAD >0) at the age of 7 years. Though the patients' age differed at the time of interview or questionnaire (7-16 years), only the period before age of 7 years was evaluated, when the whole cohort was assessable. The mean SCORAD at the age of 7 years was 28.15 ± 14.34 in the group of children who showed persistent course. To validate the predictors of persistence, an independent cohort of 20 children was established who did not belong to the study cohort. In this cohort, clinical information was obtained either via phone (n = 19) or questionnaire (n = 1), and serum collected between 2013 and 2015 was analyzed. The validation cohort consisted of 18 Caucasian and two Asian patients.

The study design was approved by the Ethics Committee of the Faculty of Medicine of the Technical University of Munich (408/17S), and written informed consent was obtained from all patients' parents. The complete questionnaire with all clinical variables assessed can be found in the online repository of this manuscript.

2.1.2 | Independent cohort

The sub-cohort from the Munich study center of the LISA (Influence of Life-style related factors on the development of the Immune System and Allergies in East and West Germany) study, a German population-based birth cohort study, has been used as an independent cohort for validation. In the present analysis, only children from the Munich study center who had parent-reported, physician-diagnosed AD in the first 3 years of life were included. In all these patients, follow-up information about the disease course was available until the age of 7 years (LISA Munich; n = 168). Further information and variables used from the LISA Munich study have been listed in the online repository of this manuscript.

2.2 | Cytokine and chemokine analyses

We measured 33 cytokines, chemokines, and growth factors in the patients' serum (Table S1) using the Bio-Plex Pro Human Cytokine 27-plex Assay (Bio-Rad Laboratories), according to the manufacturer's recommendations.

2.3 | IgE

Total serum IgE (IU/mL) and specific IgE (KU/L) to the common allergens egg white, peanut, soybean, cow's milk protein, carrot, wheat, banana, and inhalant screen (*Dermatophagoides pteronyssinus*, cat dander, dog dander, *Phleum pratense*, rye grass, *Cladosporium herbarum*, birch pollen, and *Artemisia vulgaris*) were measured using ImmunoCAP 250 (Thermo Fisher Scientific) and IMMULITE 2000 XP*i* (Siemens). For the measurement of serum IgE in the LISA cohort, see the online repository of this manuscript. Extrinsic allergic AD was characterized by serum IgE levels >100 IU/mL and/or the presence of specific IgE, whereas intrinsic nonallergic AD was defined as serum IgE levels <100 IU/mL and no detectable specific IgE.[17]

2.4 | Statistical analysis

Categorical variables are presented as frequencies and percentages; numerical values are expressed as means with standard deviation (SD) unless otherwise indicated. For each numerical variable, we used the nonparametric Mann-Whitney *U* test to test for differences between two groups—"persistence of disease" vs "nonpersistence of disease"—in both study cohort and LISA cohort, respectively. Accordingly, for each categorical variable, the chi-square test was used to analyze relationships between these two groups in both the study and LISA cohorts. Multiple testing was corrected using the Benjamini-Hochberg procedure (false discovery rate = 0.1). Results are presented as odds ratios with corresponding 95% confidence intervals (CIs) and raw and adjusted *P* values. All statistical analyses were conducted using GraphPad Prism 7.00 software (GraphPad Software).

2.4.1 | Cluster analysis and prediction model for disease course

To carry out both the cluster analysis and the prediction model, Python 3.6 with the sklearn module was used (http://www.python.org). Serum data were log-transformed, and continuous features were normalized to their mean and standard deviation. Features were excluded if more than 20 values were missing; below this threshold, missing values were imputed using the k-nearest neighbors (KNN) method. Ultimately, 76 categorical and continuous features were used (Table S1). To map the variance of the dataset onto two dimensions, the dimensionality of the dataset was reduced using factor analysis of mixed data (FAMD), with two components (https://github.com/MaxHalford/prince). The resulting two-dimensional dataset was clustered by the k-means method, which is an algorithm that partitions data into k-distinct, ideally nonoverlapping subgroups. We performed silhouette analysis to examine the separation distance between the resulting clusters and obtained a silhouette coefficient on a scale from −1 to + 1 with high values indicating high matching of a patient to the corresponding cluster and only poor matching to neighboring clusters. Differences in categorical or continuous features among the three clusters were analyzed using either the chi-square test followed by a Bonferroni p value correction or the Kruskal-Wallis test followed by Dunn's multiple comparisons test and Bonferroni p value correction, respectively. To this end, the statsmodels package of Python was used. For the prediction model of the disease course, the most discriminative features were selected using a genetic algorithm based on logistic regression. Persistence of disease by the age of 7 years was used as a response variable, and eleven of the remaining 75 features were extracted (Table S1). The

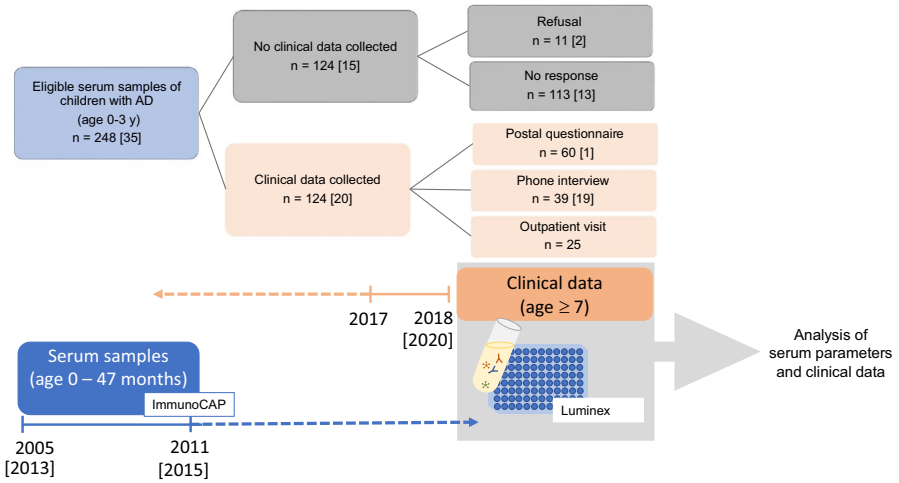


FIGURE 1 Workflow of data acquisition. Of 248 eligible serum samples, sera from 124 patients were used as clinical data could be acquired retrospectively from these patients via postal questionnaires, phone interviews, and ambulatory visits. Serum was analyzed using Luminex and ImmunoCAP. The square brackets show the numbers for the disease predictor in the validation cohort (n = 20)

reduced dataset containing these eleven features was then fed into the XGBoosting method (Extreme Gradient Boosting), which is a decision-tree-based ensemble algorithm, to classify the data into "persistence of disease at the age of 7 years" as a response variable.[18] The model was trained on 82 randomly selected patients and tested with the remaining 42 patients of the study cohort. To account for imbalance in the training dataset, which showed a ratio of 1:1.7 in children showing remission/persistence of disease at the age of 7 years, the Synthetic Minority Over-sampling Technique (SMOTE) was used on the data before training the classifier.[19] 5-fold cross-validation was consecutively applied to optimize the hyperparameters for the classifier. Using an independent second cohort (validation cohort; n = 20), the performance of the classifier was validated.

3 | RESULTS

3.1 | Data collection and characterization of the study cohort

A total of 135 of 248 patients with a physician-confirmed diagnosis of AD at the age of 0-3 years were contacted. In all patients, serum samples had been taken between 0 and 47 months of life. Ultimately, 124 of these 135 patients were willing to participate in the study via questionnaire, phone interview, and outpatient visit (study cohort) (Figure 1). Clinical information comprising family history and disease history were collected retrospectively (for all variables assessed, see Table S1; for the complete questionnaire, see the online repository). Persistent AD course was assumed when parents reported visible AD skin lesions (SCORAD >0) at the age of 7 years. Serum proteins and IgE levels were measured in the serum. Within the study cohort of 124 patients, the mean age at onset was 6.9 ± 7.6 months, and the severity of AD at onset was on average moderate, as defined by the mean SCORAD of 36.8 ± 17.3. Seventy-eight out of 124 patients (62.9%) showed persistence of AD at the age of 7 years, and 76.4% of patients suffered from an extrinsic variant of AD characterized by serum IgE levels >100 IU/mL and/or the presence of specific IgE.

3.2 | Cluster analysis and characterization of the AD clusters

First, we performed factor analysis of mixed data involving the 76 variables (Table S1; Figure 2) followed by unsupervised clustering on the condensed data using the k-means method. The elbow point in the inertia graph (Figure 2A) depicts the optimal number of three clusters (Figure 2B). A highly separating cluster configuration was reached with an average silhouette coefficient of 0.57 (Figure 2C).

Forty-two patients (33.9%) were allocated to cluster 1, 45 to cluster 2 (36.3%), and 37 to cluster 3 (29.8%; Figure 2D, Table S2). Cluster 1 was characterized by the highest percentage of patients with a positive family history of atopic diseases (85.7% vs 71% in cluster 2 and 48.6% in cluster 3; $P = .0016$). Moreover, cluster 1 patients were characterized by the lowest age at AD onset (3.5 months vs 8.0 months in cluster 2 and 9.3 months in cluster 3; $P < .0001$), the highest SCORAD at onset (42.8 vs 35.3 in cluster 2 and 31.7 in cluster 3; $P = .0095$), and the highest rate of AD persistence at the age of 7 years (73.1% in cluster 1% vs 66.7% in cluster 2 and 45.9% in cluster 3; $P = .0306$). In addition, trigger factors of AD exacerbation, such as stress, change in weather (temperature, humidity), pollen exposure, infection, and vaccination were reported more often in cluster 1 than in clusters 2 and 3. For example, stress was reported in 64.3% of patients in cluster 1, but only played a significant role in 35.6% of patients in cluster 2 and in 32.4% of patients in cluster 3 ($P = .0058$; Table S2). Moreover, allergic rhinoconjunctivitis (ARC) and allergic asthma were highly prevalent in cluster 1, with prevalences of 92.9% and 42.9%, respectively, while they were less prevalent in cluster 2 (ARC: 75.6%, allergic asthma: 8.9%). No patients in cluster 3 reported ARC or allergic asthma ($P < .0001$). In addition, food allergies were more prevalent in cluster 1 patients than in cluster 2 and 3 patients ($P < .0001$). Concerning serum IgE, patients from cluster 1 showed the highest level of total serum IgE (245 IU/mL vs 68.2 IU/mL and 130.3 IU/mL in clusters 2 and 3, respectively; $P = .0002$), as well as the highest levels of specific IgE to egg white ($P = .0006$), peanut ($P < .0001$), soybean ($P = .0003$), cow's milk

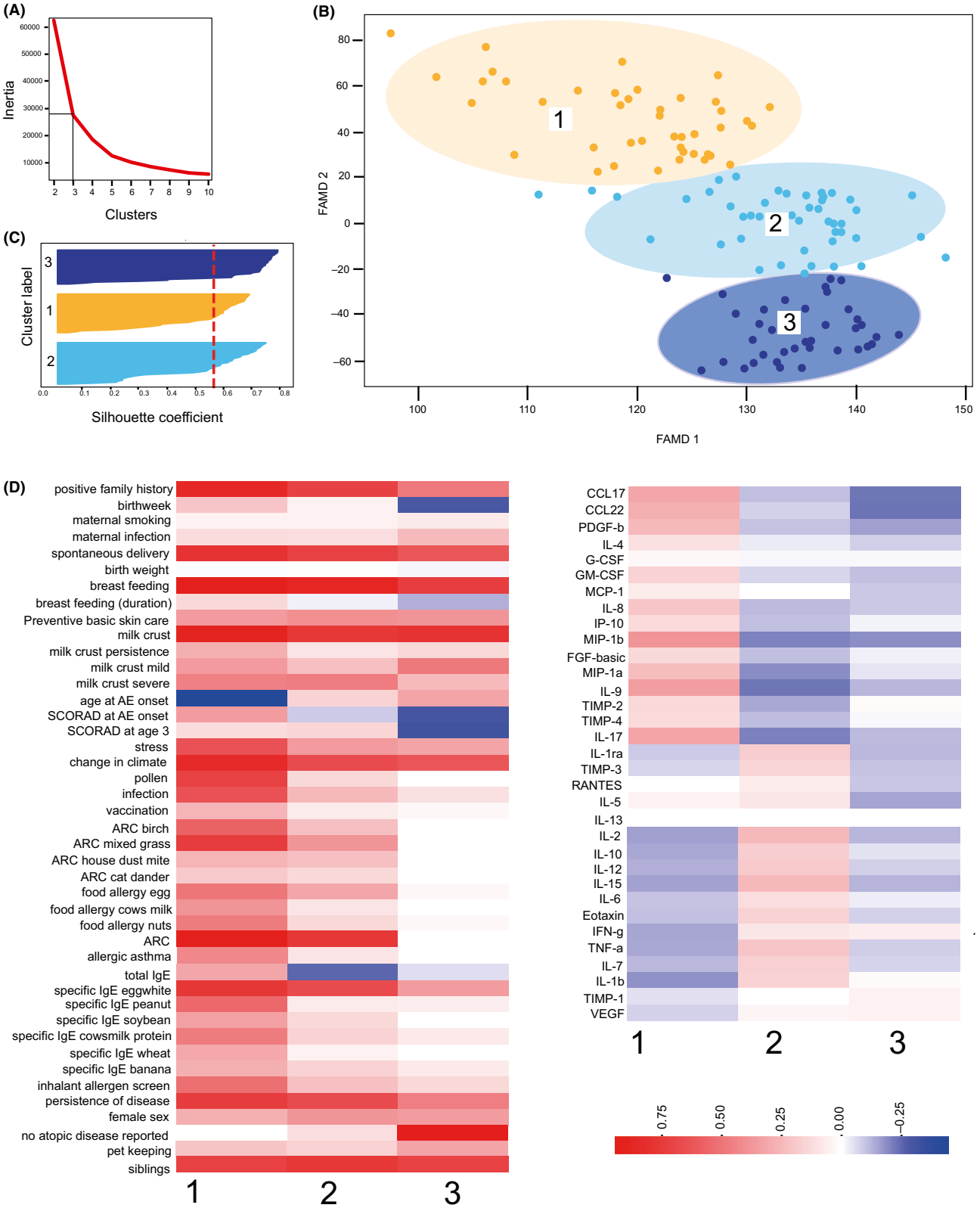


FIGURE 2 Cluster analysis revealed three distinct clusters of patients in the study cohort. As indicated by the elbow point in the inertia graph (A), unsupervised clustering approach using the k-means method revealed three distinct clusters of patients (B). The silhouette coefficient values of all patients in each cluster reached an average value of 0.57 indicating high separating quality of the clusters (C). The heat map of the three clusters shows differential expression of features within the three clusters. Red values indicate high numbers (continuous features) or percentages (categorical features), while blue values indicate low numbers (continuous features) or percentages (categorical feature) (D)

TABLE 1 Comparison of variables between the study cohort and the independent LISA Munich cohort

	Study cohort (n = 124)						LISA cohort (n = 168)					
Clinical characteristics	**noR**	**R**	**OR**	**95% CI**	***P value***	***adj. P value***	**noR**	**R**	**OR**	**95% CI**	***P value***	***adj. P value***
General information												
Female sex, no. (%)	28 (35.9)	14 (30.4)	1.28	0.59-2.79	.5347	.7615	17 (68.0)	62 (43.4)	2.78	1.13-6.85	.0227	.2043
Family history for allergic disease, no. (%)	56 (71.8)	30 (65.2)	1.36	0.62-2.97	.4428	.8325	9 (37.5)	41 (30.3)	1.38	0.56-3.40	.4882	.7323
Pets, no. (%)	16 (20.7)	12 (26.6)	0.72	0.31-1.71	.4556	.8236	2 (9.1)	18 (12.3)	0.71	0.15-3.30	.662	.7448
Siblings, no. (%)	56 (71.8)	32 (72.7)	0.95	0.42-2.18	.9122	.9527	16 (64.0)	61 (42.7)	2.39	0.99-5.77	.0482	.1735
Pregnancy												
Maternal smoking, no. (%)	4 (5.1)	3 (6.5)	0.77	0.17-3.63	.7453	.8146	4 (17.4)	13 (9.35)	2.04	0.60-6.91	.2439	.4390
Maternal infection, no. (%)	10 (12.8)	9 (20)	0.59	0.22-1.58	.2886	.7139	13 (54.2)	58 (40.8)	1.71	0.72-4.09	.2225	.4450
Birth												
Birth week, mean ± SD	39.5 ± 1.9	39.6 ± 1.5			.9188	.9388		*No premature children included*				
C-section delivery, no. (%)	24 (30.7)	12 (26.6)	1.22	0.54-2.77	.63	.8003	7 (29.1)	24 (17.0)	2.01	0.75-5.37	.1591	.3580
Birth weight, mean ± SD [g]	3305 ± 637	3454 ± 433			.2793	.7293						
Breastfeeding												
Breast-fed patients, no. (%)	69 (88.5)	36 (80)	1.92	0.67-5.25	.2009	.6745	22 (0.92)	142 (0.99)	0.08	0.01-0.89	.0092	.1656
Breastfeeding duration, mean ± SD [months]	6.5 ± 5.8	7.8 ± 7.7			.4709	.7632	*5.0 ± 1.98*	*5.19 ± 1.57*	*Only until 6 mo evaluated!*			
Milk crust												
Patients with milk crust, no. (%)	63 (82.9)	35 (76.1)	1.52	0.62-3.76	.3593	.8444	17 (68.0)	73 (51.0)	2.04	0.83-5.02	.1169	.3507
Milk crust persistence, mean ± SD [months]	11.4 ± 15.1	9.3 ± 15.2			.1926	.6963						
Milk crust severe, no. (%)	32 (43.8)	17 (38.6)	1.24	0.58-2.66	.5808	.7799						
AD												
Age at onset, mean ± SD [months]	7.6 ± 8.2	5.6 ± 6.2			.4653	.8100						
Early onset AD (≤3 mo), no. (%)	36 (46.1)	24 (52.2)	0.79	0.38-1.63	.517	.7593						
SCORAD at onset, mean ± SD	38.2 ± 16.4	34.4 ± 18.5			.1719	.6733						
SCORAD at age 3, mean ± SD	35.0 ± 18.3	14.7 ± 14.6			<.0001	.0047						
Age at remission, mean ± SD [years]	NA	3.5 ± 1.7										
Preventive basic skin care	30 (38.9)	17 (38.6)	1.01	0.47-2.17	.9719	.9719						
Trigger factor for AD exacerbation												
Stress, no. (%)	43 (57.3)	12 (27.3)	3.58	1.60-8.02	.0015	.0235						
Change in the weather, no. (%)	64 (83.1)	21 (47.7)	5.39	2.33-12.49	<.0001"/>	.0024						

(Continues)

TABLE 1 (Continued)

Clinical characteristics	Study cohort (n = 124)						LISA cohort (n = 168)					
	noR	R	OR	95% CI	*P value*	*adj. P value*	noR	R	OR	95% CI	*P value*	*adj. P value*
Pollen exposure, no. (%)	30 (39.5)	6 (13.6)	4.13	1.56-10.97	.0029	.0341						
Infection, no. (%)	30 (38.5)	12 (27.9)	1.62	0.72-3.62	.2431	.7141						
Vaccination, no. (%)	8 (10.7)	7 (16.3)	0.61	0.21-1.83	.3784	.8084						
Atopic diseases												
Allergic rhinitis, no. (%)	49 (64.5)	21 (46.7)	2.07	0.98-4.39	.0552	.4324	3 (13.6)	13 (9.6)	1.48	0.39-5.69	.5646	.7259
ARC- birch pollen, no. (%)	23 (31.1)	11 (25)	1.35	0.58-3.14	.4806	.7287						
ARC- grass pollen, no. (%)	36 (48.6)	12 (27.3)	1.20	0.53-2.75	.6614	.7971						
ARC- house dust mite, no. (%)	13 (17.6)	7 (15.9)	1.13	0.41-3.08	.8164	.8721						
ARC- cat dander, no. (%)	10 (13.5)	4 (9.1)	1.56	0.46-5.32	.4725	.7403						
Allergic asthma, no. (%)	18 (23.4)	4 (9.3)	2.97	0.94-9.46	.0561	.3767	5 (21.7)	11 (7.9)	3.23	1.01-10.38	.0395	.1778
Allergic asthma- age at onset, mean ± SD [years]	4.7 ± 3.1	2.75 ± 0.9			.3677	.8229						
Food allergy, no. (%)	31 (40.8)	15 (33.3)	1.38	0.64-2.98	.4142	.8111						
Food allergy egg, no. (%)	26 (34.2)	10 (22.2)	1.82	0.78-4.25	.1633	.6977						
Food allergy cow's milk, no. (%)	14 (18.4)	6 (13.3)	1.47	0.52-4.14	.4665	.7831						
Food allergy nuts, no. (%)	16 (21)	11 (24.4)	0.82	0.34-1.98	.6649	.7813						
No other atopic disease reported, no. (%)	21 (27.3)	19 (42.2)	0.51	0.24-1.11	.0897	.4216						
Allergic asthma and allergic rhinitis, no. (%)	16 (21)	3 (7)	3.56	0.97-13.00	.044	.4136	2 (9.1)	2 (1.5)	6.50	0.87-48.8	.0386	.2316
Serum IgE												
Serum total IgE level, mean ± SD [IU/mL]	152.3 ± 321	147.8 ± 310.5			.0883	.4611	384.1 ± 250.9	248.8 ± 247.8			.1383	.3556
Serum total IgE level >100 IU/mL, no. (%)	31 (39.7)	13 (29.5)	1.57	0.71-3.47	.26	.7188	18 (94.7)	107 (86.3)	2.86	0.36-22.85	.3013	.4930
Specific IgE ≥0.35 kU/L to egg white, no. (%)	50 (64.1)	24 (53.3)	1.56	0.74-3.23	.24	.7520	3 (60.0)	12 (60.0)	1.00	0.14-7.40	1.0000	1.0000
Specific IgE ≥0.35 kU/L to peanut, no. (%)	19 (24.7)	9 (20)	1.31	0.54-3.21	.5535	.7651	1 (0.20)	7 (35.0)	0.46	0.04-5.00	.5201	.7201
Specific IgE ≥0.35 kU/L to soybean, no. (%)	12 (15.4)	8 (17.8)	0.84	0.32-2.24	.729	.8158						
Specific IgE ≥0.35 kU/L to cow's milk protein, no. (%)	20 (25.6)	10 (22.2)	1.21	0.51-2.87	.6706	.7687	2 (0.4)	9 (0.45)	0.81	0.11-5.99	.8403	.8897
Specific IgE ≥0.35 kU/L to carrot, no. (%)	3 (5.7)	1 (3.1)	1.86	0.19-18.7	.5928	.7739						

(Continues)

TABLE 1 (Continued)

Clinical characteristics	Study cohort (n = 124)						LISA cohort (n = 168)					
	noR	R	OR	95% CI	*P value*	*adj. P value*	noR	R	OR	95% CI	*P value*	*adj. P value*
Specific IgE ≥0.35 kU/L to wheat, no. (%)	11 (14.3)	4 (9.1)	1.67	0.50-5.59	.4042	.8260						
Specific IgE ≥0.35 kU/L to banana, no. (%)	15 (19.2)	7 (15.9)	1.26	0.47-3.37	.6468	.8000						
Specific IgE ≥ 0.35 kU/I to common pollen And environmental inhalants, no. (%)	28 (35.9)	9 (20)	2.24	0.94-5.32	.0641	.3766	3 (15.8)	15 (12.1)	1.36	0.36-5.24	.6514	.7817
Serum cytokines												
CCL17, mean ± SEM [pg/mL]	53 883.57 ± 22 819.53	35 891.71 ± 22 073.59			.3742	.4749						
CCL22, mean ± SEM [pg/mL]	56 984.24 ± 17 881.31	80 108.62 ± 29 443.81			.0499	.4117						
TIMP-1, mean ± SEM [pg/mL]	643 708.96 ± 23 004.99	662 732.14 ± 30 182.07			.3596	.4747						
TIMP-2, mean ± SEM [pg/mL]	391 224.26 ± 12 398.69	391 292.69 ± 21 124.54			.4120	.4856						
TIMP-3, mean ± SEM [pg/mL]	32 949.17 ± 1463.21	54 824.46 ± 20 880.4			.3403	.4679						
TIMP-4, mean ± SEM [pg/mL]	1308.37 ± 177.96	1231.99 ± 272.27			.3359	.4819						
IL-9, mean ± SEM [pg/mL]	171.03 ± 4.93	164.09 ± 6.19			.1861	.4387						
IP-10, mean ± SEM [pg/mL]	867.373 ± 85.72	931.21 ± 125.53			.2076	.4282						
PDGF-b, mean ± SEM [pg/mL]	4094.00 ± 160.95	4105.21 ± 323.56			.2313	.4017						
MIP-1b, mean ± SEM [pg/mL]	234.91 ± 15.42	219.72 ± 15.07			.2980	.4470						
RANTES, mean ± SEM [pg/mL]	19 024.30 ± 624.89	17 229.02 ± 668.82			.0576	.2715						
IL-1b, mean ± SEM [pg/mL]	5.35 ± 0.74	4.67 ± 0.8			.2605	.4298						
IL-1ra, mean ± SEM [pg/mL]	112.26 ± 15.56	121.7 ± 24.69			.4845	.5158						
IL-2, mean ± SEM [pg/mL]	8.76 ± 2.55	14.13 ± 5.41			.1736	.4407						
IL-4, mean ± SEM [pg/mL]	6.17 ± 0.19	6.01 ± 0.36			.2196	.4026						
IL-5, mean ± SEM [pg/mL]	6.24 ± 0.75	4.62 ± 0.81			.0519	.2855						
IL-6, mean ± SEM [pg/mL]	15.68 ± 3.66	15.16 ± 5.33			.1505	.4515						
IL-7, mean ± SEM [pg/mL]	12.38 ± 1.33	11.79 ± 2.34			.0358	1.0000						
IL-8, mean ± SEM [pg/mL]	83.45 ± 50.60	29.9 ± 4.85			.0502	.3313						
IL-10, mean ± SEM [pg/mL]	26.84 ± 6.50	29.66 ± 10.66			.2083	.4043						
IL-12, mean ± SEM [pg/mL]	48.92 ± 5.86	49.52 ± 8.69			.4160	.4734						
IL-13, mean ± SEM [pg/mL]	8.14 ± 1.08	8.63 ± 1.75			.4742	.5216						
IL-15, mean ± SEM [pg/mL]	6.05 ± 2.37	13.62 ± 5.78			.2642	.4152						
IL-17, mean ± SEM [pg/mL]	90.44 ± 4.55	79.75 ± 5.54			.0638	.2339						

(Continues)

TABLE 1 (Continued)

	Study cohort (n = 124)						LISA cohort (n = 168)					
Clinical characteristics	noR	R	OR	95% CI	*P value*	*adj. P value*	noR	R	OR	95% CI	*P value*	*adj. P value*
Eotaxin, mean ± SEM [pg/mL]	108.54 ± 6.36	96.77 ± 7.51			.1427	.4709						
FGF-basic, mean ± SEM [pg/mL]	84.01 ± 2.97	76.34 ± 3.41			.0585	.2413						
G-CSF, mean ± SEM [pg/mL]	88.455 ± 5.67	81.17 ± 6.65			.1557	.4282						
GM-CSF, mean ± SEM [pg/mL]	121.37 ± 13.50	105.19 ± 14.79			.3930	.4803						
INF-g, mean ± SEM [pg/mL]	102.42 ± 14.18	76.36 ± 8.9			.0403	.6650						
MCP-1, mean ± SEM [pg/mL]	125.17 ± 25.48	104.89 ± 20.29			.4928	.5082						
MIP-1a, mean ± SEM [pg/mL]	4.78 ± 0.24	4.38 ± 0.25			.1959	.4310						
TNF-a, mean ± SEM [pg/mL]	94.59 ± 14.74	94.1 ± 20.55			.4948	.4948						
VEGF, mean ± SEM [pg/mL]	71.20 ± 8.61	103.61 ± 14.87			.0489	.5379						

Note: Categorical variables are shown as counts and percentages. Continuous variables are shown as mean ± SD or SEM, as indicated. Odds ratios (OR) including 95% confidence intervals (CI) indicate association of variables with course of disease. R, remission at the age of 7 y. noR, no remission at the age of 7 y, persistence of disease.

protein ($P < .0001$), wheat ($P < .0001$), and common pollen and environmental inhalants ($P < .0003$). Analysis of serum cytokines revealed higher levels of IL-9 in cluster 1 than in cluster 3 ($P = .0417$). The levels of serum IL-17 ($P = .0101$) and MIP-1b ($P = .0156$) were higher in cluster 1 than in clusters 2 and 3. Cluster 2 was characterized by the highest levels of 14 serum cytokines as compared to the other clusters. Notably, there was a trend of higher amounts of IL-1R, IL-2, IL-15, and TNF-α in cluster 2. In cluster 3, all serum cytokines broadly showed the lowest or second lowest levels among all clusters apart from VEGF, TIMP-1, and IL-13, which had the highest serum levels in cluster 3, although the difference was not statistically significant. The numbers and significance levels of all features are listed in Table S2.

3.3 | Analysis of factors correlating with an increased risk of disease persistence at the age of 7 years

Cluster 1 was characterized by the severest AD endotype, as indicated by the highest SCORAD at disease onset, the highest prevalence of other atopic diseases, the highest rate of positive family history, and the highest rate of persistent disease course. Therefore, we sought to identify factors associated with disease persistence until the age of 7 years. To validate the findings of our study cohort (n = 124), we identified corresponding variables in the independent LISA Munich cohort (n = 168) and analyzed them for their association with persistent disease course (Table 1). Although 62.9% of children showed a persistent disease course in the study cohort, only 14.9% (n = 25) in the LISA Munich cohort did so. In both cohorts, increased odds ratios (ORs) for persistence were observed for female sex (raw $P = .0227$ in the LISA cohort), positive family history, cesarean section rate, milk crust, allergic rhinitis and/or allergic asthma (raw $P = .0386$ in LISA, $P = .044$ in our cohort), and increased IgE levels. However, none of these associations reached statistical significance after correction for multiple testing (Table 1). In contrast, significant associations with disease persistence were found for the trigger factors exposure to pollen (OR = 4.13, CI = 1.56-10.97, adjusted $P = .0341$), change in weather (OR = 5.39, CI = 2.33-12.49, adjusted $P = .0024$), or stress (OR = 3.58, CI = 1.6-8.02, adjusted $P = .0235$), which were not assessed in the LISA Munich cohort. Moreover, SCORAD at the age of 3 years was significantly higher in the persistent group (35.0 ± 12.26) than in the nonpersistent group (12.67 ± 14.58; adjusted $P = .0047$). To gain a deeper insight into the phenotype of children with AD in both the persistent and nonpersistent groups, we assessed lesion distribution at both disease onset and at the age of 3 years (Table 2, Figure 3). While a high percentage of patients in both groups showed involvement of the face and limbs at the time of disease onset, significant differences were observed between the groups at the age of 3 years. More patients in the persistent than in the nonpersistent group showed involvement of the upper limbs (OR = 6.48, CI = 2.61-16.05, $P = .0014$), lower limbs (OR = 5.03, CI = 2.1-12.03, $P = .0014$), and flexures (OR = 2.68,

TABLE 2 Distribution of skin lesions in both the persistent and non-persistent groups at the age of onset , at the age of 3 y, and at the age of 7 y

	Study cohort (n = 124)					
Clinical phenotype	**noR**	**R**	**OR**	**95% CI**	***P* value**	***adj. P* value**
Distribution of lesions at onset						
Face, no. (%)	49 (74.2)	27 (73)	1.07	0.43-2.66	.8882	.9565
Neck, no. (%)	12 (18.1)	7 (18.9)	0.95	0.34-2.68	.9263	.9263
Upper limbs, no. (%)	50 (75.8)	27 (73)	1.16	0.46-2.9	.7549	.9608
Lower limbs, no. (%)	39 (59.1)	24 (64.9)	0.78	0.34-1.8	.564	.8773
Abdomen, no. (%)	20 (30.3)	9 (24.3)	1.35	0.54-3.82	.5175	.9056
Back, no. (%)	19 (28.8)	5 (13.5)	2.59	0.88-7.64	.0785	.2748
Flexures, no. (%)	30 (54.5	15 (40.5)	1.22	0.54-2.76	.6295	.8813
Distribution of lesions at the age of 3 y						
Face, no. (%)	21 (31.8)	8 (21.6)	1.73	0.68-4.43	.25	.5000
Neck, no. (%)	15 (23.1)	5 (13.5)	1.92	0.64-5.9	.2421	.5649
Upper limbs, no. (%)	53 (81.5)	15 (40.5)	6.48	2.61-16.05	<.0001	.0014
Lower limbs, no. (%)	49 (75.4)	14 (37.8)	5.03	2.10-12.03	.0002	.0014
Abdomen, no. (%)	8 (12.3)	4 (10.8)	1.16	0.32-4.14	.8215	.9584
Back, no. (%)	6 (9.2)	1 (2.7)	3.66	0.42-31.67	.2099	.5877
Flexures, no. (%)	42 (64.6)	15 (40.5)	2.68	1.17-6.14	.0186	.0868
Distribution of lesions at the age of 7 y						
Face, no. (%)	15 (23.1)	0 (0)				
Neck, no. (%)	12 (18.4)	0 (0)				
Upper limbs, no. (%)	53 (81.5)	0 (0)				
Lower limbs, no. (%)	52 (80)	0 (0)				
Abdomen, no. (%)	6 (9.2)	0 (0)				
Back, no. (%)	8 (12.3)	0 (0)				
Flexures, no. (%)	40 (61.5)	0 (0)				

Note: Variables are shown as counts and percentages. Odds ratios (OR) including 95% confidence intervals (CI) indicate association of variables with course of disease. R, remission at the age of 7 y. noR, no remission at the age of 7 y, persistence of disease.

CI = 1.17-6.14, $P = .0868$; Table 2, Figure 3). Interestingly, the observed differences were not correlated with the application of topical steroids (never/proactive/if needed; $P = .5587$) or basic therapy with emollients (never, daily, multiple times a week; $P = .9456$). Apart from involvement of the face (31.8% of patients showed lesions in the face at the age of 3 years vs 23.1% at 7 years), patients within the persistent group presented similar lesion distribution at the ages of 3 and 7 years (Table 2, Figure 3).

Concerning serum cytokines, no significant differences were found between the groups after correction for multiple comparisons. However, the raw *P* values for increased levels of IL-7 and IFN-γ were lower in the persistent group than in the nonpersistent group, and there were lower levels of CCL22 and VEGF in the persistent group than in the nonpersistent group (Figure S1, Table 1). In addition, no significant correlations were found between any of the cytokines and SCORAD at the ages of 3 or 7 years (data not shown).

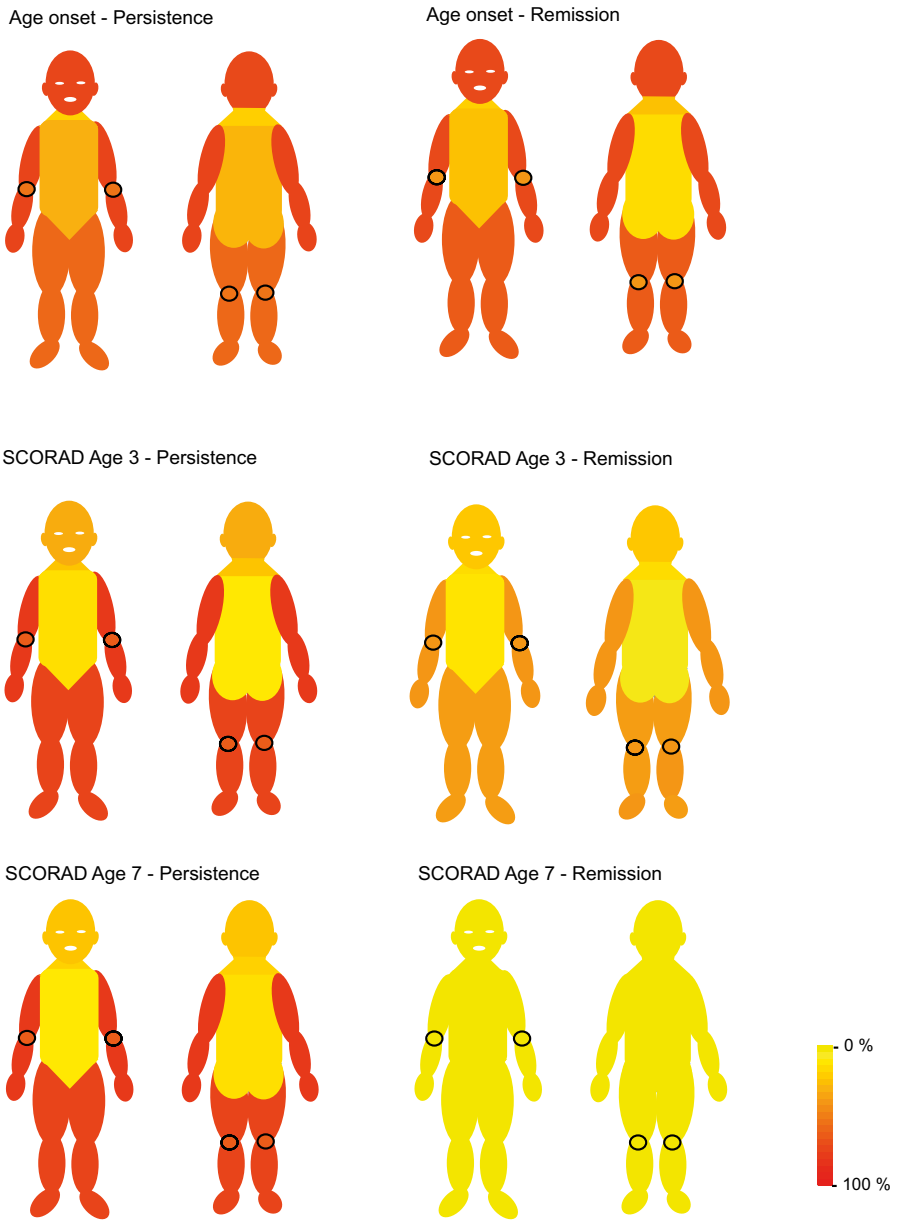


FIGURE 3 Distribution of AD lesions in both the persistent and nonpersistent groups at the age of onset (upper row), at the age of 3 y (middle row), and at the age of 7 y (lower row). Red colors indicate a high percentage of patients showing involvement of the indicated site, and yellow colors indicate low percentages of patients showing involvement of that site. Flexural sites are shown in circles

3.4 | A logistic regression-based model to predict disease persistence

To assess the impact of clinical variables and serum proteins in combination, we next sought to establish a classifier predicting disease outcome. To this end, we performed feature selection using a genetic algorithm with logistic regression. Eleven of the remaining 75 variables were extracted, among which the following variables had the highest impact for disease classification: "SCORAD at age 3," "VEGF," "IL-17," "persistence of milk crust," "breastfeeding" and the trigger factors "change in weather" and "pollen" (Figure 4A, Table S1). Consistent with both the cluster analysis and the correlation of variables with disease persistence, "SCORAD at age 3," the trigger factors "change in weather" and "pollen," and to a lesser extent breastfeeding, were positively associated with disease persistence, whereas higher levels of VEGF were positively correlated with remission. In addition, absence of milk crust correlated positively with disease remission in this prediction model.

Next, XGBoost was used to establish a classifier to predict disease outcomes. Disease persistence at the age of 7 years was defined as the response variable. For each patient, the probability of disease persistence until the age of 7 years was calculated. The classifier was trained using 82 randomly selected patients of the study cohort (28 patients with remission at the age of 7 years (R), 54 with persistence at the age of 7 years (noR); mean SCORAD at onset: 37.946 ± 18.99) and tested on 42 patients of the study cohort (18 patients with R, 24 with noR, mean SCORAD at onset: 35.29 ± 15.86). With a cutoff probability value of >55% for clear prediction for persistence and of <45% for remission, as well as an AUC of 0.997, the classifier diagnosed all patients in the training cohort correctly, apart from one patient who could not be given a clear prediction (probability of persistence = 54.13%;

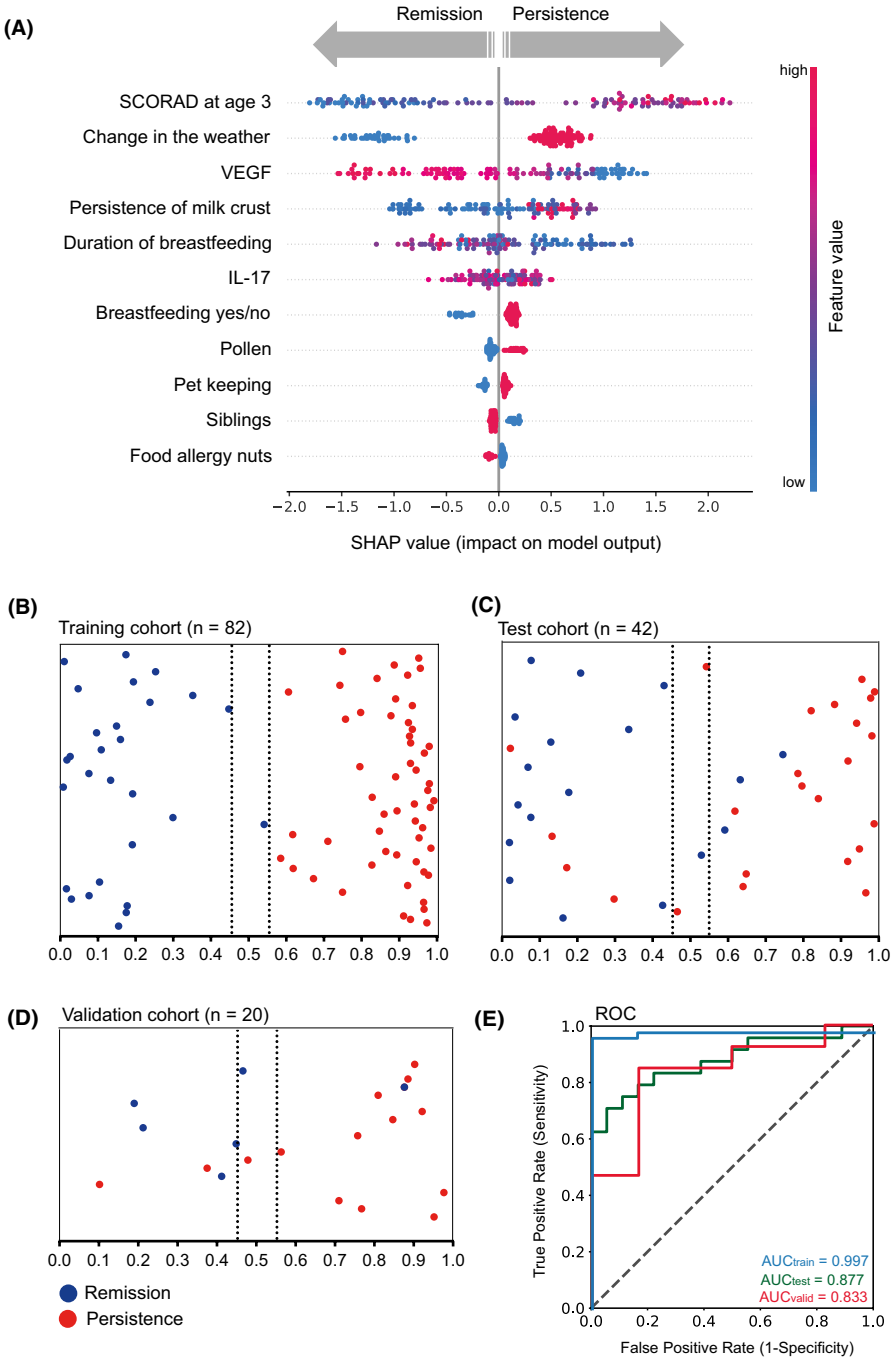


FIGURE 4 Prediction model for persistence/remission of disease by the age of 7 y. Eleven of the 75 variables used for prediction are shown. Red dots on the right side (left side) from the middle line indicate that high values of the respective feature (eg, high SCORAD) show a positive (negative) association with the target variable "persistence," while blue dots on the right side (left side) from the middle line indicate that low values of the feature show positive (negative) association with the target variable "persistence" (A). The classifier for persistent disease course was trained in a cohort of 82 patients with persistent (red dots) and nonpersistent (blue dots) disease course (B). The classifier was then applied in a test cohort of 42 patients (C) and in an independent validation cohort of 20 patients (D). Prediction probabilities for persistence of disease are indicated on the lower axis of each graph. Dotted lines indicate the area of unclear prediction. The AUCs of all three cohorts are shown in the ROC curve (E)

Figure 4B,E). In the test cohort, 32 patients were correctly classified, seven were misclassified, and three had probabilities ranging from >45% to <55% and could not be definitively classified. Thus, the disease predictor delivered a sensitivity of 81.8% and a specificity of 82.4% for persistence of disease, with an AUC of 0.877 (Figure 4C,E). Next, we sought to validate the performance in a second independent validation cohort of 20 patients: six with R and 13 with noR; mean SCORAD at onset: 58.58 ± 15.67. Fifteen of the 20 patients were correctly classified, while three were misclassified and two had probabilities ranging from >45% to <55% and could not be classified (Figure 4D). In line with the test cohort, a sensitivity of 84.6% and a specificity of 80.0% for disease persistence was reached, with an AUC of 0.833 (Figure 4E). The probabilities of disease persistence in each patient are listed in Table S3.

4 | DISCUSSION

Atopic dermatitis is a highly heterogeneous disease.[5,20,21] Based on clinical presentation and current diagnostic tools, clinicians cannot predict whether the individual disease course will be self-limiting.

In the present study, we discovered three endotypes of AD in infancy. Cluster 1 represented the severe endotype: children with

an early disease onset, high SCORAD, allergic comorbidities, and elevated serum IgE, IL-17, and MIP-1b. Cluster 3 was characterized by a benign endotype: late disease onset, lower SCORAD values, few allergic comorbidities, and low concentrations of inflammatory serum proteins, apart from VEGF, TIMP-1, and IL-13. However, cluster 2 represents an intermediate endotype.

From an immunological point of view, cluster 1 (high serum IgE, Th2, and Th17 cytokines) resembled the cytokine signature of adult AD described by Brunner et al.[11] The elevated levels of IL-17 might be linked to bacterial colonization and barrier defects, as infiltrating Th2/IL-17 cells of acute AD lesions release IL-17 when activated by the *Staphylococcus aureus* superantigen.[22] In line with this observation, and given that cluster 1 was characterized by the lowest age of disease onset, Thijs et al found that patients with early AD onset and "profoundly inflamed" skin had high IL-17 serum levels.[14] Cluster 3 had the lowest serum cytokine values, indicating that systemic inflammation was not as pronounced as in the other patient groups. Interestingly, cluster 3 showed a trend toward the highest VEGF, TIMP1, and IL-13 levels. IL-13 is a typical Th2 cytokine that is clearly associated with AD.[11,23] Several studies have found elevated VEGF in the serum of adults with AD, but the correlation to disease severity is unclear.[24-26] In the present study, high levels of VEGF were associated with a higher rate of disease remission, indicating that patients in cluster 3 have better prognosis. Reduta et al demonstrated that TIMP1, which has cell growth-promoting activity,[27] shows higher levels in the serum samples of patients in remission from acute contact dermatitis than in patients with active contact dermatitis,[28] indicating that it plays a role in disease cessation. In line with this, patients in cluster 3 had the lowest SCORAD values at disease onset and almost no allergic comorbidities. Cluster 2 showed a trend toward increased levels of IL-1R, IL-2, IL-15, and TNF-α, while classical Th2-related serum cytokines did not increase. In accordance with the clinical presentation, showing an intermediate endotype between cluster 1 (severe) and cluster 3 (mild), this immunological signature represented mixed inflammation, with upregulation of innate (IL-1R, TNF-α) and adaptive immunity (IL-2, IL-15). Hence, the intermediate cluster 2 may comprise more endotypes that could only be characterized in a larger cohort.

The concept of AD endotypes is a new approach to address disease heterogeneity. Transcriptome analysis and serum biomarker detection may allow researchers to identify patient subgroups that are responders or nonresponders to certain therapies.[11,14,20,29] Thijs et al defined AD endotypes in adults based on serum cytokine signature.[14] In that study, the authors detected four distinct clusters of patients: clusters 1 and 3 were associated with a severe clinical presentation, while clusters 2 and 4 presented a milder endotype. In the present study, clustering resulted in three patient subgroups. Although serum AD markers in childhood differ from those of adult AD,[30] certain analogies are apparent. For instance, cluster 2 as described by Thijs et al had the highest frequency of childhood onset and the lowest VEGF values. Moreover, VEGF, which is a potent regulator of angiogenesis,[31] enhances the Th1 phenotype, thus inhibiting polarization of the AD-driving Th2 cells.[32] In line with these findings, we observed that children with high serum VEGF levels had a higher chance of disease remission by the age of 7 years. In the next step, we assessed the prognostic value of clinical and serum parameters. Previous studies have proposed several other predictors of chronic disease in adults, such as high serum IgE levels, high eosinophil count, and *filaggrin* null mutations.[6,33-35] However, most studies have only investigated a single or a few factors, while studies examining a multitude of clinical variables and serum proteins in the same cohort of children are rare. In addition, research has mostly focused on biomarkers that correlate with the current disease score, not on whether serum proteins can predict disease progression. By calculating the ORs for disease persistence, we demonstrated that certain clinical characteristics of the endotype clusters were associated with disease persistence. Results were obtained from our cohort and an independent population-based birth cohort (LISA Munich). Both showed an association between disease persistence and allergic disorders. In a recent study, Berna et al performed an unsupervised clustering analysis of seven clinical variables in a cohort of more than 8000 children suffering from AD in the USA. They observed five distinct clusters, which differed in terms of race, family income, and family history. As in our study, the authors were able to assign each cluster to a certain risk of AD persistence, thus affirming the concept of high- and low-risk disease endotypes.[36] Among the individual factors, allergies were associated with a higher rate of persistence, as in our study. Next, we found that SCORAD at the age of 3 years was significantly higher in the group with persistent AD, indicating that chronification may be associated with high SCORAD and that it may occur very early. This argument was corroborated by Celakovska et al,[37] who showed that SCORAD reflected the duration of atopic dermatitis lesions. In addition, Czarnowicki et al demonstrated that circulating CLA^+ T_{EM} and T_{CM} cells were highly correlated with AD severity, indicating that memory for inflammation and thus risk for chronification may be higher in more severely affected individuals.[38] Interestingly, in contrast to adults, where head and neck involvement has been associated with persistent AD,[39] we showed a significant association between persistence of disease and involvement of the upper and lower limbs, as well as the flexures. In addition to the LISA Munich cohort, we assessed subjective trigger factors for disease exacerbation. In this regard, we observed a significant association between disease persistence and the trigger factors change in weather, exposure to pollen, and stress. In accordance with the nasal and bronchial mucosal symptoms in patients with asthma and rhinitis,[40,41] changes in weather, such as humidity and temperature, may exacerbate "hyperreactivity of the skin." In addition, the trigger factor "stress" is of high importance, as it warrants potential therapeutic interventions. Indeed, disease management programs integrating the biological and psychological aspects of AD are highly beneficial for young patients and parents.[42]

High-risk children must be identified, as potential prevention strategies are available to improve individual disease course, such as daily emollient use or proactive topical treatment,[43-45] as well

as an increasing number of targeted AD therapies (biologics).[46,47] Therefore, we tested the combination of clinical and serological parameters for their prognostic value concerning disease persistence using a genetic algorithm alongside logistic regression. Our disease predictor correctly classified patients with a sensitivity and specificity of >80% in both the test and validation cohorts. Factors with a high impact on prediction of disease persistence were SCORAD at the age of 3 years, trigger factor change (weather and pollen), milk crust, breastfeeding, and low VEGF. These factors could be easily evaluated in clinical practice.

Our study had some limitations. Firstly, we collected our data retrospectively, which may have led to missing or incorrect data. Second, we only assessed the disease course until the age of 7 years and had no information about potential disease recurrence at later time points. Furthermore, we cannot rule out selection bias caused by the monocentric study design: Severe cases may have predominated at the university department, which may explain the higher rate of children with persistent course in our cohort than in the literature, as well as the rate of persistence in the LISA Munich cohort.[2] Moreover, we used patient- or caregiver-reported outcomes, which can contribute to a higher persistence rate.[48] It was for this reason that the findings for the factors characterizing disease persistence were validated in an independent cohort (LISA). In addition, the robustness of the disease predictors was inferred not only by cross-validation but also by challenging its performance in a separate validation cohort established 2 years after the study cohort was built. Common trends regarding the risk factors for persistence were found in both the LISA Munich cohort and our own, despite the different rates of persistent disease course between the two studies. This strengthens our findings. Despite all these limitations, the present study can be regarded as proof of concept, demonstrating that stratification of individual risk factors and prediction of disease progression can be performed using large datasets of complex and heterogeneous inflammatory diseases, such as childhood AD. We investigated a so-far unsolved clinical problem, namely whether children will develop chronic AD, to emphasize that systematic assessment of clinical and serological variables, combined with innovative bioinformatic approaches, is useful to overcome relevant medical needs.

In summary, we demonstrated that children with AD can be stratified into three distinct endotypes based on clinical parameters and inflammatory blood signatures. We propose a classifier that discriminates persistent course from remission, which could be transferred into clinical practice, allowing individualized risk assessment and precision medicine.

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CONFLICT OF INTEREST

None of the authors have a conflict of interest in relation to this work.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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