Confirmation of multiple endotypes in atopic dermatitis based on serum biomarkers

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Background: Atopic dermatitis (AD) is a highly heterogeneous disease, both clinically and biologically, whereas patients are still being treated according to a "one-size-fits-all" approach. Stratification of patients into biomarker-based endotypes is important for future development of personalized therapies.
Objective: Our aim was to confirm previously defined serum biomarker-based patient clusters in a new cohort of patients with AD.
Methods: A panel of 143 biomarkers was measured by using Luminex technology in serum samples from 146 patients with severe AD (median Eczema Area and Severity Index = 28.3; interquartile range = 25.2-35.3). Principal components analysis followed by unsupervised k-means cluster analysis of the biomarker data was used to identify patient clusters.
A prediction model was built on the basis of a previous cohort to predict the 1 of the 4 previously identified clusters to which the patients of our new cohort would belong.
Results: Cluster analysis identified 4 serum biomarker–based clusters, 3 of which (clusters B, C, and D) were comparable to the previously identified clusters. Cluster A (33.6%) could be distinguished from the other clusters as being a "skin-homing chemokines/IL-1R1–dominant" cluster, whereas cluster B (18.5%) was a "$T_H1/T_H2/T_H17$-dominant" cluster, cluster C (18.5%) was a "T_H2/T_H22/PARC-dominant" cluster, and cluster D (29.5%) was a "T_H2/eosinophil-inferior" cluster. Additionally, by using a prediction model based on our previous cohort we accurately assigned the new cohort to the 4 previously identified clusters by including only 10 selected serum biomarkers.
Conclusion: We confirmed that AD is heterogeneous at the immunopathologic level and identified 4 distinct biomarker-based clusters, 3 of which were comparable with previously identified clusters. Cluster membership could be predicted with a model including 10 serum biomarkers. (J Allergy Clin Immunol 2021;147:189-98.)

Key words: *Atopic dermatitis, endotypes, clusters, biomarkers, prediction, personalized medicine, principal components analysis*

Atopic dermatitis (AD) is 1 of the most common chronic inflammatory skin diseases worldwide and is characterized by a diverse clinical manifestation and a highly heterogeneous pathophysiology.[1] Classically, AD is stratified into different disease phenotypes according to clinical characteristics, including age, age of onset, ethnicity, and presence of other atopic diseases such as allergic rhinitis and asthma.[2,3] Despite the highly heterogeneous character of the disease, there are currently no endotype-specific published data for any licensed drug for the treatment of AD in Europe or the United States. Therefore, the current treatment guidelines for AD could not consider disease subtypes, resulting in a high unmet need for individualized treatment options.

In the past decade more and more advances have been made in characterizing the immunologic differences underlying AD. Although AD is considered to be a primarily T_H2 cell–driven disease, it has now become clear that T_H1, T_H17, and pathways are likely to contribute to AD pathogenesis as well.[4-6] Because of the heterogeneity T_H22 cytokine of the disease, it is unlikely that novel molecular therapies targeting specific immunologic pathways will be equally effective in all patients with AD, which makes stratification of subtypes of patients with AD of increasingly important. Identification of patients by relevant and validated biomarkers is a prerequisite for a more personalized therapeutic approach.[7] Nevertheless, the distinct molecular mechanisms driving different disease subtypes of AD, previously defined as endotypes[8], are as yet inadequately described.

In a recently published study, we identified 4 clearly differentiated clusters of patients by using a data driven approach based on 147 biomarkers measured in 193 patients with moderate-to-severe AD.[9] Each cluster was characterized by a specific serum biomarker profile, implying that a distinct underlying immunopathologic pathway drives each cluster. Two of these clusters were characterized by a T_H2-dominated biomarker profile,[9] suggesting that the patients in these clusters would be ideal candidates for T_H2-inhibiting therapies, such as the recently

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*These authors contributed equally to this work.
Research was funded by Regeneron and Sanofi Genzyme Pharmaceuticals, Inc.
Disclosure of potential conflict of interest: M.S. de Bruin-Weller is a principal investigator and advisory board member for AbbVie and Regeneron Pharmaceuticals, Inc; a principal investigator, advisory board member, and consultant for Sanofi Genzyme; an advisory board member for Eli Lilly and UCB; and a principal investigator and advisory board member for Pfizer. The rest of the authors declare that they have no relevant conflicts of interest.
Received for publication January 10, 2020; revised April 7, 2020; accepted for publication April 24, 2020.
Available online June 8, 2020.
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0091-6749

https://doi.org/10.1016/j.jaci.2020.04.062

Abbreviations used
AD: Atopic dermatitis
CsA: Cyclosporine A
EASI: Eczema Area and Severity Index
EGF: Epidermal growth factor
IQR: Interquartile range
PARC: Pulmonary and activation-regulated chemokine
PCA: Principal components analysis
TARC: Thymus and activation-regulated chemokine
TNFSF: Tumor necrosis factor superfamily
TSLP: Thymic stromal lymphopoietin

developed anti–IL-4Rα mAb dupilumab or the anti–IL-13 antibodies tralokinumab and lebrikizumab. Stratification of patients into distinct endotypes might contribute to the development of personalized medicine approaches and precision-based care in the future. However, the previously defined patient clusters still need to be replicated in an independent patient population. The aim of the present study was to confirm the previously identified clusters of patients with AD based on distinct serum biomarker profiles by using the same data-driven approach on a new cohort of patients with severe AD. Additionally, we aimed to build a prediction model enabling stratification of patients into 1 of the 4 previously defined clusters by using a small set of selected markers, which might be incorporated in clinical trials or standard practice as a convenient tool to identify endotypes in the future.

METHODS

Patients and samples

To confirm the endotypes on the basis of a clinically well-defined large cohort of patients with severe AD, who are most eligible for systemic/biologic treatments, we used data on a previously reported cohort of patients with AD who were selected on the basis of AD severity (Eczema Area and Severity Index [EASI] score >21) and treated only with topical corticosteroids, the original aim of which was to predict the need for systemic therapy.[10] Of the 152 patients in this cohort, 6 were also included in the cohort from the study of Thijs et al[9]; to lower the risk of bias, these 6 patients were excluded from the cohort for the current study, resulting in 146 patients included in the current study. Clinical characteristics were retrospectively extracted from the patients' electronic medical records. For the current study, AD severity was assessed by using EASI score, according to the Harmonizing Outcomes Measures in Eczema recommendations.[11] Severity scores from the previous cohort[9] were measured before the availability of these recommendations and were assessed by using the Six Area, Six Sign Atopic Dermatitis (SASSAD) severity score, which was the standard severity score in our center at that time. Both severity scores incorporate grading of AD signs and assessment of body region involvement. All patients were diagnosed with AD according to the criteria of Hanifin and Rajka.[12] The protocols of this study were approved by the institutional review board of the University Medical Center Utrecht (The Netherlands), adhering to the principles of the Declaration of Helsinki.

Serum biomarkers

The biomarker levels of a panel of 143 serum biomarkers were measured by using Luminex technology[13] and an in-house validated panel of analytes listed in Table E1 (see this article's Online Repository at www.jacionline.org). The same biomarker measurements as previously published[10] were used in the current study. Baseline thymus and activation-regulated chemokine (TARC)/C-C motif chemokine (CCL) 17 (CCL17) levels, which are currently the best-performing and accepted biomarker for disease severity,[14] were measured during routine care by using Quantikine ELISA immunoassays (R&D Systems, Inc, Minneapolis, Minn).

Statistical methods

Replication of the 4 distinct patient clusters. Principal components analysis (PCA) followed by unsupervised k-means cluster analysis of the serum biomarker data was used to identify patient clusters. Additionally, PCA followed by k-means cluster analysis was repeated on the pooled serum biomarker data of the current cohort and the original cohort from the study of Thijs et al.[9] Clinical characteristics and averages of serum biomarkers were analyzed between the clusters by using 1-way ANOVA for normally distributed variables, the Kruskal-Wallis test for nonnormally distributed variables, and the chi-square test for percentages.

Prediction model based on previously defined clusters. We built a prediction model based on the biomarker data used in the study of Thijs et al[9] to predict the 1 of the 4 previously identified clusters to which the patients in our cohort would belong. The prediction model was built by using a supervised random forest approach (the package randomForest[15] in R). The importance of each biomarker in the classification of patients was estimated by using the mean decrease in accuracy. Prediction model accuracy, defined as the fraction of correctly predicted cases (1 – model error rate), was studied for several prediction models by using all 140 of the markers common to the 2 cohorts, as well as the top 70, top 20, and top 10 biomarkers. A flowchart presenting all the steps of the prediction model can be found as Fig E1 (in this article's Online Repository at www.jacionline.org).

Statistical analyses were performed by using R Project software, version 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria),[16] and SPSS software for Windows, version 21.0 (IBM Inc, Armonk, NY). Serum biomarker data were normalized by Box-Cox transformation before analyses. Before replication of the cluster analysis and building of the prediction model, we merged the serum biomarker data of both data sets and corrected them for batch effects related to 2 different batches (the package sva[17] in R). *P* values lower than .05 were considered statistically significant.

RESULTS

Patient characteristics and cluster replication

Of the 146 patients, 56% were male. Their median age was 30.5 years (IQR = 22.0-50.0), their median EASI score was 28.3 (IQR = 25.2-35.3), and their median TARC/CCL17 level was 4192 pg/mL (IQR = 2088-8496); all were treated with topical corticosteroids at the moment of sampling. Patient characteristics from the current study and original study[9] are summarized in Table I. Disease severity in the current cohort differed from that in the original cohort,[9] in which patients with moderate-to-severe AD (median TARC/CCL17 level = 1766 pg/mL [IQR = 874-5215]) were included ($P < .001$). Besides, the current cohort had a significantly higher percentage of patients who had been treated with any systemic immunosuppressive drug (not including systemic corticosteroids) within 1 year before sampling (22.6% vs 11.4%, respectively [$P = .010$]). Age, sex, atopic comorbidities, and age of onset did not significantly differ between the 2 cohorts.

A total of 143 serum biomarkers were determined via multiplex immunoassay. After PCA on the Box-Cox–transformed serum biomarker data set, the cumulative percentage of variance showed that 90% of the data set's variance was described by the first 48 principal components (Fig 1). The first 48 principal components were included in the unsupervised k-means cluster analysis, resulting in the identification of 4 distinct patient clusters (clusters A, B, C, and D, which are displayed in terms of the first 3 principal components in Fig. 2, *A*). The cluster membership per patient was

TABLE I. Baseline characteristics

Clinical characteristic	Current cohort (n = 146)	Original cohort (n = 193)
Age (y), median (IQR)*	30.5 (22.0-50.0)	30.0 (21.0-42.0)
Male, no. (%)	81 (56)	81 (42)
EASI score, median (IQR)	28.3 (25.2-35.3)	NA
SASSAD score, median (IQR)	NA	31.6 (23.0-37.5)
Serum TARC/CCL17 level, median (IQR)	4191.5 (2087.5-8495.5)	NA
Atopic disease, no (%)		
Allergic asthma	80 (55)	88 (46)
Allergic rhinitis	95 (65)	124 (64)
Food allergy	59 (40)	NA
No atopic disease	25 (17)	50 (26)
Missing data	1 (1)	3 (2)
Age of onset, no. (%)		
0-1 y	58 (38)	88 (46)
2-11 y	64 (44)	74 (38)
12-18 y	4 (3)	8 (4)
>18 y	10 (7)	15 (8)
Missing data	10 (7)	8 (4)
Hospitalization for AD, no. (%)	64 (44)	NA
History of immunosuppressive drug use <1 year before sampling, no. (%)†	22 (11)	33 (23)

NA, Not available.
Categoric variables are presented as counts and percentages; continuous variables are presented as medians (IQRs).
*Age at time of sample collection.
†Including azathioprine, cyclosporin A, methotrexate, enteric-coated mycophenolate sodium, mycophenolate mofetil, and tacrolimus.

added back into the complete data set, and clinical characteristics were compared between the 4 clusters (Table II). The averages of the serum biomarker levels were calculated per cluster to characterize the biomarker profiles driving the 4 clusters (Fig 2, *B* and see Table E2 in this article's Online Repository at www.jacionline.org) and were also compared with the previously identified clusters[9] (reported as clusters 1, 2, 3, and 4 [Fig 2, *B*]).

Cluster A represented 33.6% of the patients with AD. The median age in this cluster was 35.0 years (IQR = 22.5-51.0), and the median EASI score was 28.0 (IQR = 25.3-35.6). Cluster A was distinct from clusters C and D by having higher levels of C-C chemokines (CTACK/CCL27, TARC/CCL17, MDC/CCL22, and RANTES/CCL5) and IL-1R1 ("skin-homing chemokines/IL-1R1–dominant" cluster). Regarding clinical characteristics, the percentage of patients who were treated with any systemic immunosuppressive drug (not including systemic corticosteroids) within 1 year before sampling was significantly higher in cluster A than in the other clusters (37% vs 18%, 11%, and 16, respectively [$P = .043$]). Cluster A was the only cluster that did not correspond to any of the previously defined clusters.[9]

Cluster B represented 18.5% of the patients with AD. The median age in this cluster was 25.0 years (IQR = 20.0-50.0), and the median EASI score was 25.2 (IQR = 23.0-31.4). Cluster B was characterized by a high inflammatory state distinctive from that of the other clusters by virtue of having the highest levels of T_H2-related (IL-4, IL-5, and IL-13), T_H1-related (IFN-γ, TNF-α, and TNF-ß), T_H17-related (IL-17 and IL-21), and epithelial-related (IL-25, IL-33, and TSLP) cytokines (the "$T_H1/T_H2/T_H17$-dominant" cluster), as shown in Fig 2, *B*. The biomarker profile of cluster B was comparable to that of the previously defined cluster 4.

Cluster C represented 18.5% of the patients with AD; their median age was 32.0 years (IQR = 22.0-55.0). The patients in this cluster had significantly higher severity scores than did the patients in clusters A, B, and D (median EASI = 37.8 [IQR = 28.2-44.6]) ($P = .001$). Cluster C was uniquely defined by high levels of T_H2-related cytokines (pulmonary and activation-regulated chemokine [PARC], IL-13, IL-5, eotaxin, and eotaxin-3), IL-22, and IL-33 (the "T_H2/T_H22/PARC-dominant" cluster). The biomarker profile of cluster C resembled that of the previously identified cluster 1.

Cluster D represented 29.5% of the patients with AD; they had a median age of 32.0 years (IQR = 23.0-48.0) and a median EASI score of 27.4 (IQR = 25.6-32.8). It was characterized by a relatively low inflammatory state particularly distinctive from that of the other clusters by virtue of having low serum levels of T_H2/severity-related (MDC, PARC, and TARC) and eosinophil-related markers (RANTES, eotaxin, and eotaxin-3) (the "T_H2/eosinophil-inferior" cluster). The biomarker profile of cluster D resembled the profile of the previously identified cluster 2.

Other clinical characteristics, including age, sex, atopic comorbidities, age of onset, and hospitalization rate, did not differ significantly between the 4 clusters (Table II and Fig 2, *C*).

In addition, we analyzed the merged data sets (the previously published and present data sets) by using a PCA and k-means cluster analysis, and here again we identified 4 patient clusters. The biomarker profiles of the merged clusters were largely comparable with those of the original patient clusters.[9] "Merged cluster" 1 was characterized by the lowest levels of epithelial cytokines and IL-1–related cytokines; merged cluster 2 was characterized by the highest levels of T_H2 family cytokines, IL-1–related cytokines, and TSLP; merged cluster 3 was characterized by the highest levels of T_H2 family cytokines and pulmonary and activation-regulated chemokine (PARC/CCL18); and merged cluster 4 was characterized by the lowest levels of IFN-α and apelin. Of the patients who clustered together in the original clusters, 88.3% clustered together again in 1 of the merged clusters (see Table E3 in this article's Online Repository at www.jacionline.org). For the replication cohort, 68.5% of the patients clustered together again in 1 of the merged clusters.

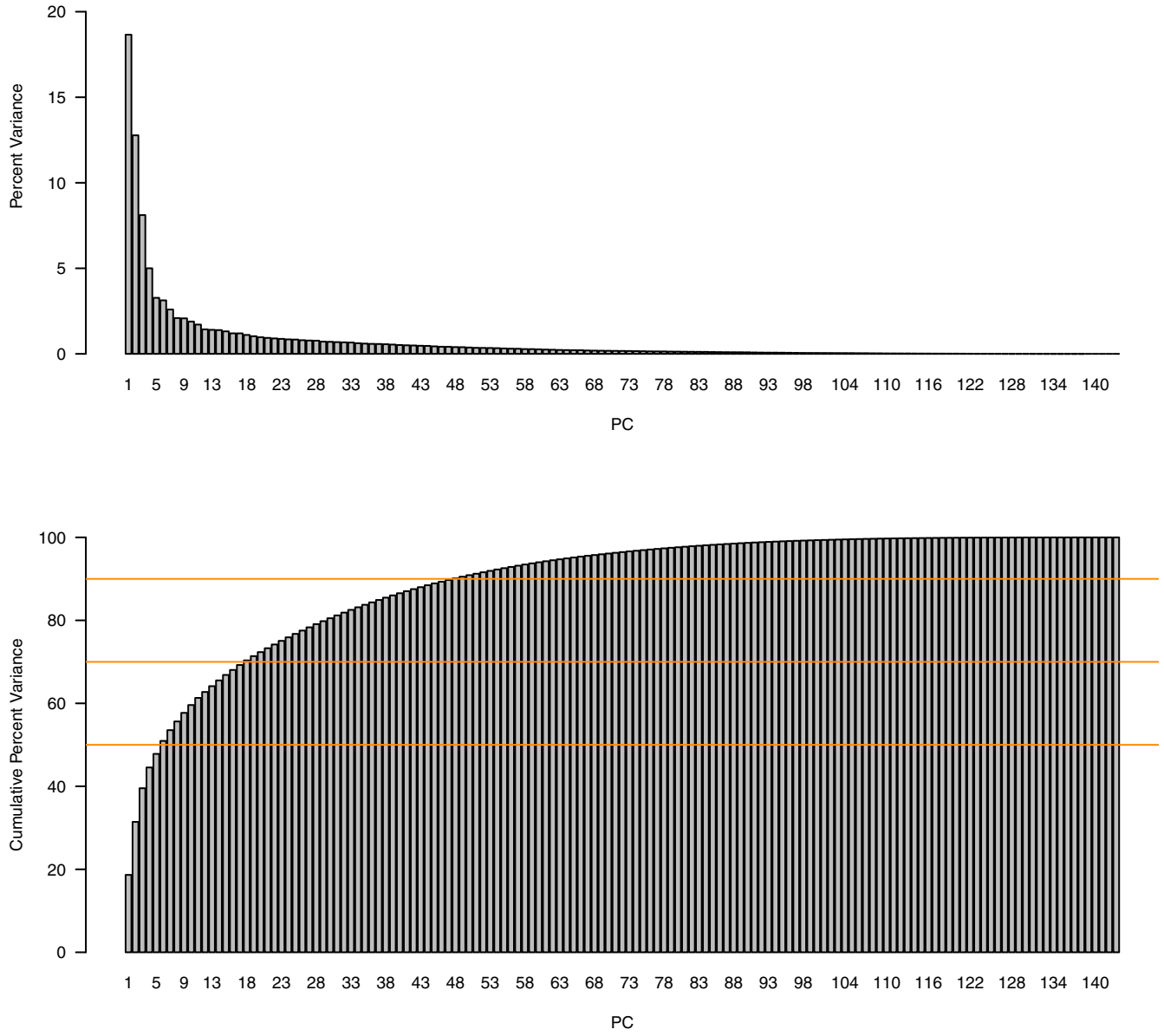

FIG 1. Variance described by principal components. The first 6 principal components (PCs) describe 50% of the variance, and the first 48 PCs describe 90% of the variance in the Box-Cox–normalized serum biomarker data set.

Cluster prediction

As we could (re)confirm 3 of the 4 previously defined patient clusters, we next used a supervised random forest approach along with the biomarker data of Thijs et al[9] to build a prediction model of cluster membership (cluster 1, 2, 3, or 4) for the patients of the current cohort. Only biomarkers determined in both data sets were used for this analysis, resulting in a total of 140 serum biomarkers. Biomarkers were sorted by importance for prediction based on the mean decrease in accuracy. The different steps of this analysis are described in Fig E1.

The top 10 biomarkers were IL-37, IL-1rα, XCL-1, eotaxin/CCL11, IL-1ß, IL-26, LIGHT/tumor necrosis factor superfamily (TNFSF)14, IL-1R1, epidermal growth factor (EGF), and TSLP (see Table E4 in this article's Online Repository at www.jacionline.org). The accuracy of the prediction model based on the original study[9] including all 140 biomarkers was 94.1% (see Fig E2 in this article's Online Repository at www.jacionline.org) and the out-of-bag estimate of error rate (ie, number of incorrect classifications) was 5.3% (Table III). When only the top 10, top 20, and top 70 biomarkers were included, the rates of accuracy were 86.7%, 90.4% and 93.6%, respectively. The out-of-bag estimates of error rate were 13.8%, 9.6%, and 5.3%, respectively (see Tables E5-E7 in this article's Online Repository at www.jacionline.org).

We then applied the models to the current data set and used the cluster membership of the model including all 140 markers as a reference. We compared this membership with the membership predicted by including only the top 10, top 20, and top 70 markers. The accuracy of the model was 87.0% when the top 70 biomarkers were included versus 73.3% and 81.5% with inclusion of the top 10 and the top 20, respectively.

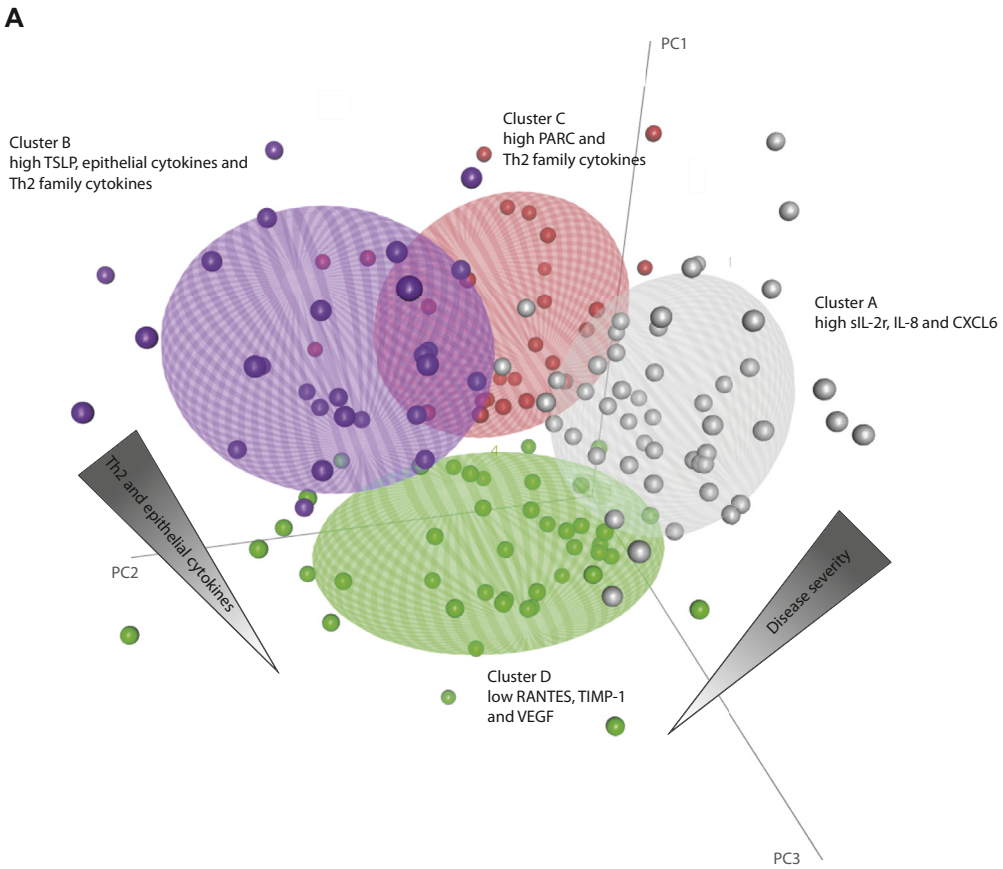

FIG 2. Comparison of clusters identified in the original and replication cohorts. **A,** Using unsupervised k-means clustering of the first 48 principal components (PCs) resulted in the identification of 4 patient clusters (A, B, C, and D). All 146 patients are presented in a 3-dimensional plot in terms of the first 3 PCs. Colors and colored ellipses represent clusters. PC1 explained 18.7% of the variance, PC2 explained 12.8% of the variance, and PC3 explained 8.1% of the variance. Clinical characteristics were analyzed between the 4 clusters (Table II). A difference between the clusters was found only in disease severity. Averages of serum analytes were calculated and compared per cluster to characterize cluster's unique biomarker profiles. **B**, Averages of Box-Cox–transformed serum biomarker data per cluster of the replication cohort were compared with the previously defined clusters 1, 2, 3, and 4. Radar plots show selected markers that were significantly higher or lower expressed in 1 of the clusters compared with the other clusters. The biomarker profile of cluster B resembled the profile of the previously identified cluster 4, cluster C resembled cluster 1, and cluster D resembled cluster 2. **C**, Clinical characteristics per cluster of the replication cohort were compared with the previously defined clusters 1, 2, 3, and 4. Box plots represent medians with IQRs: the upper whisker extends to the largest value no further than 1.5*IQR from the third quartile, whereas the lower whisker extends to the smallest value at 1.5*IQR to the first quartile. Disease severity was assessed by using the SASSAD severity score in the original cohort and by using EASI score in the current cohort. Both severity scores include only gradation of AD signs and body region involvement and do not take patient-reported outcomes into account. The maximum value for the SASSAD severity score is 108, and the maximum value for the EASI is 72. Disease severity was relatively higher in clusters 1 and 3, and A and B than in the other clusters. Radar plots show that no differences in other clinical characteristics between the patient clusters in both cohorts were found.

DISCUSSION

Because of the development of new therapies for AD targeting different cytokine pathways, the stratification of patients into endotypes driven by distinct molecular pathways is of increasing importance to moving toward more personalized medicine. In a recent study we classified patients with AD into 4 serum biomarker–based clusters that could represent endotypes.[9] By using the same data-driven approach, the current study once more identified 4 patient clusters with a distinct profile of serum biomarkers in a different cohort of patients with severe AD. Regarding their biomarker profiles, 3 of the 4 clusters (representing 66.4% of the patients) were similar to the previously identified clusters. Endotyping of patients with AD may contribute to precision medicine by allowing treatment to be tailored for individual patients, and it may be important to better inform which patients are most likely to benefit from specific targeted therapies.[18]

Because 3 of the 4 clusters were confirmed in the current study, we were able to further characterize and name the clusters by their discriminating biomarker profiles. Patients stratified into cluster B were characterized by a relatively high inflammatory state and

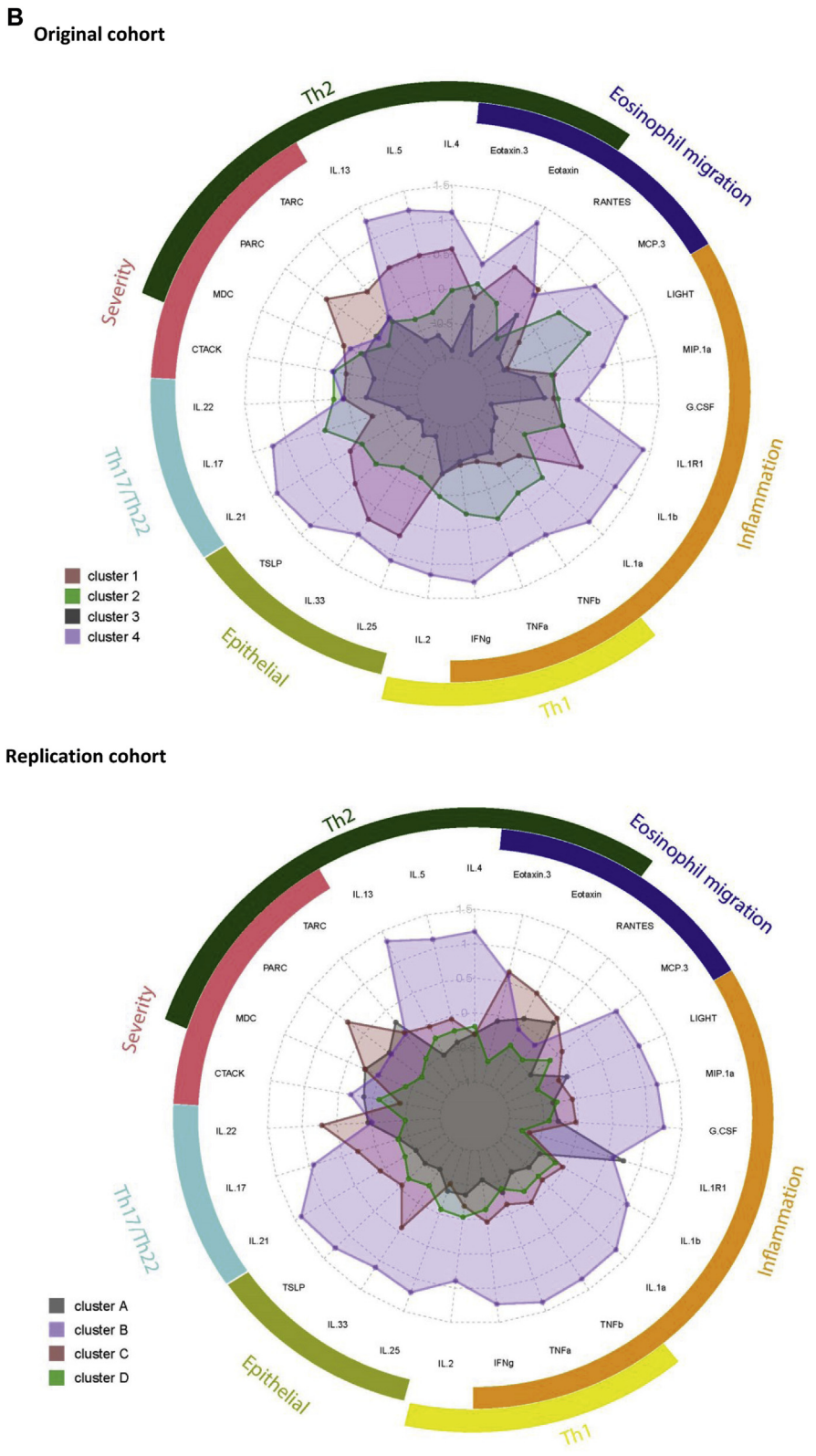

FIG 2. *(Continued.)*

could be distinguished from the other clusters as being the $T_H1/T_H2/T_H17$-dominant cluster. Cluster C shared relatively high T_H2-related cytokine levels with the $T_H1/T_H2/T_H17$-dominant cluster, although it separated itself from the other clusters by showing high levels of IL-22 and PARC. Cluster D was characterized by the lowest levels of eotaxins, RANTES, PARC, MDC, TARC, and IFN-α and was thereby defined as the T_H2/eosinophil-inferior cluster. Patients identified in cluster A showed higher

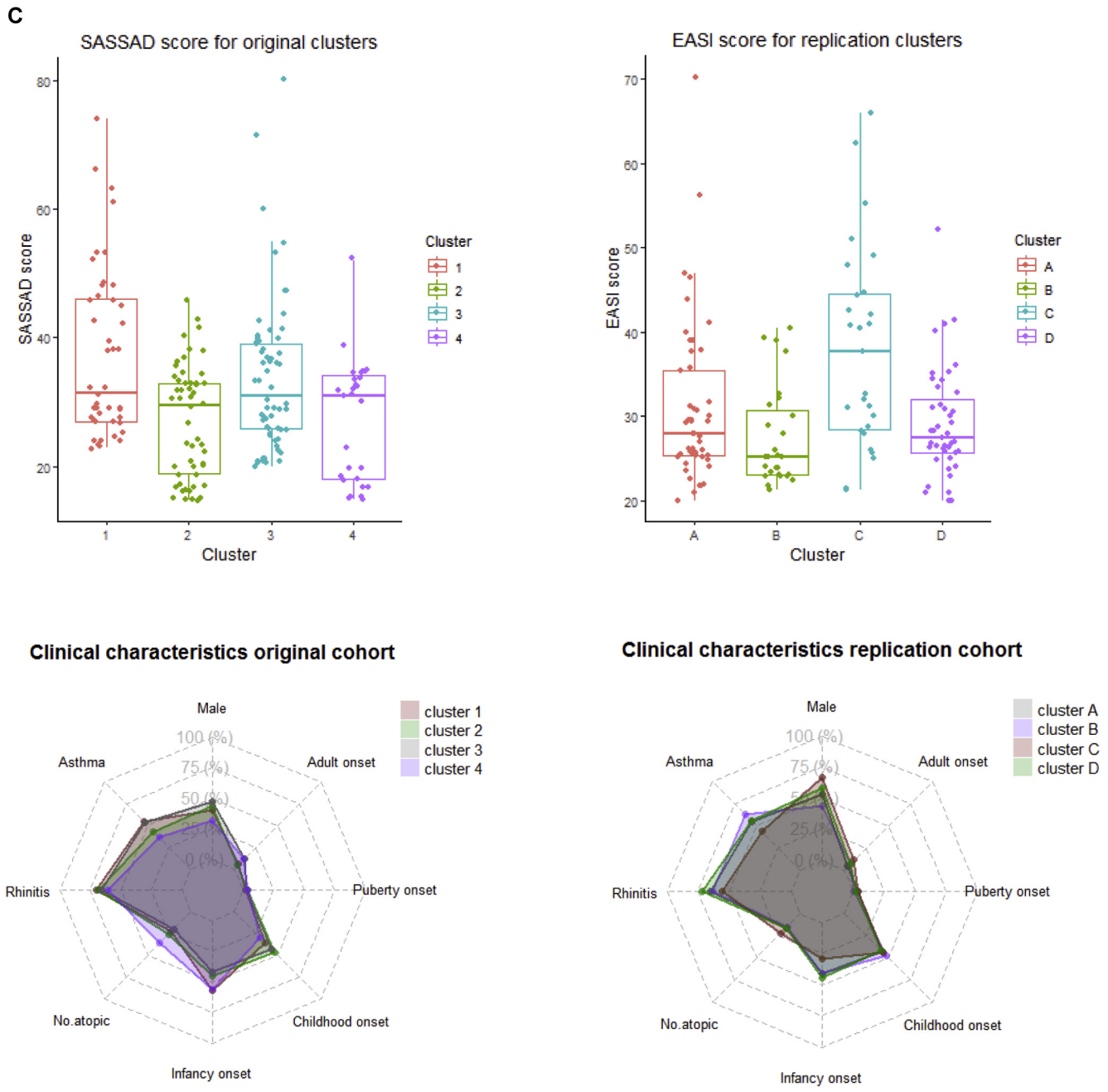

FIG 2. *(Continued.)*

levels of the C-C chemokines CTACK/CCL27, TARC/CCL17, MDC/CCL22, and RANTES/CCL5. CTACK/CCL27, TARC/CCL17, and MDC/CCL22 are known to bind the C-C chemokine receptors CCR10 and CCR4,[19,20] respectively, thereby enabling skin-specific homing of CD4+ T-cells.[21] RANTES/CCL5 is a ligand for CCR3 and CCR5 and is considered to maintain eosinophilic infiltration in chronic inflammation of AD skin.[22] Furthermore, patients in cluster A showed higher expression of serum IL-1R1 levels. Previous studies have shown that serum IL-1R1 levels are significantly increased in patients with AD compared with in healthy controls[23] and that the upregulation of IL-1R1 is associated with frequent exacerbations in patients with asthma.[24] On the basis of the underlying biomarker profile, cluster A could be defined as the skin-homing chemokines/IL-1R1–dominant cluster.

Although cluster D showed the lowest expression of several severity-related markers (TARC, PARC, and MDC), this was not reflected by the lowest EASI score. Similar to in the previous cohort, the 4 identified clusters of patients with AD in the present study were clinically distinguished by disease severity, with the T_H2/T_H22/PARC-dominant cluster showing a significantly higher EASI score. However, because all patients had high lesion/sign scores, we consider this difference as not being clinically relevant. Furthermore, we were unable to identify an association between

TABLE II. Clinical characteristics for the 4 clusters of patients with AD

Clinical characteristic	Cluster A (n = 49)	Cluster B (n = 27)	Cluster C (n = 27)	Cluster D (n = 43)	*P* value
Patients with AD (%)	33.6	18.5	18.5	29.5	
Age (y), median (IQR)*	35.0 (22.5-51.0)	25.0 (20.0-50.0)	29.0 (22.0-55.0)	32.0 (23.0-48.0)	.535
Male, no. (%)	26 (53)	12 (44)	18 (67)	25 (58)	.401
EASI score, median (IQR)	28.0 (25.3-35.6)	25.2 (23.0-31.4)	37.8 (28.2-44.6)	27.4 (25.6-32.8)	**.001**
TARC baseline (pg/mL), median (IQR)	5024 (2816-11750)	3501 (1388-11500)	4142 (2068-16000)	3278 (1787-5430)	**.037**
Atopic disease, no. (%)					
Allergic asthma	27 (55)	17 (63)	12 (44)	24 (56)	.582
Allergic rhinitis	32 (65)	17 (63)	15 (56)	31 (72)	.285
Food allergy	22 (45)	12 (44)	6 (22.2)	19 (44)	.238
No atopic disease	8 (16)	4 (15)	6 (22)	7 (16)	.852
Age of onset, no. (%)					
0-1 y	20 (41)	11 (41)	8 (29)	19 (44)	.955
2-11 y	21 (43)	13 (48)	12 (44)	18 (42)	
12-18 y	2 (4)	0	1 (4)	1 (2)	
>18 y	2 (4)	2 (7)	3 (11)	3 (7)	
Missing data	4 (8)	1 (4)	3 (11)	2 (5)	
Hospitalization for AD, no. (%)	22 (45)	13 (48)	12 (44)	17 (40)	.522
History of immunosuppressive drug use <1 y before sampling, no. (%)†	18 (37)	5 (18)	3 (11)	7 (16)	**.043**

Categoric variables are presented as counts and percentages; continuous variables are presented as medians (IQRs). Boldface indicates statistical significance.
*Age at time of sample collection.
†Including azathioprine, cyclosporine A, methotrexate, enteric-coated mycophenolate sodium, mycophenolate mofetil, and tacrolimus.

TABLE III. Model accuracy for predictive model including all 140 biomarkers

	Original cluster				
Predicted cluster	1	2	3	4	Class error
1	41	0	3	0	6.8%
2	2	51	1	0	5.6%
3	2	0	60	0	3.2%
4	0	2	0	26	7.1%

Confusion matrix showing the accuracy of the model built on the original data set of 140 overlapping serum biomarkers measured in 193 patients with moderate-to-severe AD, used to predict cluster membership in the current cohort of 146 patients with severe AD. The out-of-bag estimate rate of accuracy for the model was 5.3%.

the 4 clusters and other clinical characteristics, including age of onset or atopic comorbidities. This result highlights the challenges in identifying patient subgroups based only on clinical features and might indicate that individualized treatment options should be based not on clinical phenotypes of AD but instead on biomarker-based endotypes.

One of the 4 clusters identified in the current study (ie, the skin-homing chemokines/IL-1R1–dominant cluster A) could not be matched with any of the previous clusters of the study by Thijs et al.[9] An explanation for the different fourth cluster could be the difference in the percentage of patients who used immunosuppressive drugs within 1 year before sampling, as this percentage was higher in the current cohort than in the original cohort; moreover, it was significantly higher in the noncorresponding skin-homing chemokines/IL-1R1–dominant cluster A. The majority of these patients were treated with cyclosporine A (CsA) in the year before sampling. CsA is a calcineurin inhibitor that selectively acts on T cells through inhibiting signal transduction mediated by T-cell receptor activation.[25] It has been shown previously that CsA treatment in patients with AD suppresses the levels of IL-2–, IFN-γ–, and IL-4/IL-5/IL-13–producing T cells and T-cell products, including TARC/CCL17.[26-28] However, data on the long-term effects of CsA treatment on serum biomarkers after discontinuation are lacking. In agreement with previous findings in CsA-treated patients with AD, patients in the skin-homing chemokines/IL-1R1–dominant cluster A showed the lowest serum levels of IFN-γ, IL-4, IL-5, and IL-13.

The ability to endotype patients based on serum biomarkers has already been demonstrated in asthma, where anti–IL-13, anti–IL-4/IL-13, and anti–IL-5 therapies appeared to be more effective in T_H2-dominant patient groups. Clinical trials investigating the efficacy of treatment with the anti–IL-4/IL-13 receptor mAb dupilumab in patients with AD showed that only 35% to 40% of the patients achieved clear or almost clear skin,[29-31] which corresponds to the percentages of patients in the $T_H1/T_H2/T_H17$-dominant and T_H2/T_H22/PARC-dominant clusters. On the other hand, the numbers of nonresponders to dupilumab treatment among patients with AD are very low,[30,32] indicating that T_H2 cytokines might not be the most relevant markers to discriminate patients with AD in this overall T_H2-high population. This might also explain why the top 10 biomarkers based on the mean decrease in accuracy of our prediction model that were found to be distinctive for the 4 clusters did not include any T_H2-related markers. Although 2 clusters shared a relatively T_H2-low cytokine profile compared with the other 2 clusters, these patients still express higher levels of T_H2-related cytokines compared with levels that have previously been reported for healthy controls.[9,23] Prediction of treatment response by serum biomarker profiles in patients with AD is scarce. A single phase 2b study investigating treatment with tralokinumab (anti–IL-13) suggested that baseline serum DPP-4 levels, reflecting IL-13 activity, might serve as a predictive biomarker for patients with AD who may benefit from tralokinumab treatment.[33] Furthermore, a phase 2a study of IL-22 blockade with fezakinumab showed that patients with higher baseline expression of IL-22 had greater disease improvement during fezakinumab treatment,[34] although IL-22 expression was measured in skin biopsy specimens, which is hard to implement in daily practice. Theoretically, patients in our $T_H2/T_H22/$

PARC-dominant cluster might be optimal candidates for anti–IL-22 treatment.

Both the original study and the current study made use of a panel of more than 140 serum biomarkers to confirm the presence of 4 endotypes within patients with AD. Although, for the implementation of serum biomarker–based endotypes in clinical trials and daily practice, a smaller set of markers is desired. In the current study we built a prediction model (based on the biomarker data used in the study of Thijs et al[9]) that could be used to classify our patients into 1 of the 4 original clusters with good accuracy, even when using only the top 10 biomarkers (IL-37, IL-1rα, XCL-1, eotaxin/CCL11, IL-1ß, IL-26, LIGHT/TNFSF14, IL-1r1, EGF and TSLP). Surprisingly, none of those markers are T_H2-related cytokines, but they consisted of IL-1–, IL-10–, and epithelium-related markers. We hence hypothesize that markers related to other cytokine pathways might be (more) important to defining endotypes in an overall T_H2-dominant disease such as AD. In the future, application of such a prediction model, resulting in a small panel of biomarkers, might enhance tailored decision making in the management of patients with moderate-to-severe AD and might contribute to more personalized medicine. However, use of the prediction model should be tested in longitudinal studies and randomized controlled trials with drugs targeting specific pathways first.

A limitation of the study is that the current cohort was not completely independent from the original cohort because it included patients from the same center with comparable demographic characteristics. The previous study by Thijs et al[9] included patients with moderate-to-severe AD with a broad range of severity scores, whereas our cohort included only patients with severe AD, which makes it more difficult to generalize the results for the complete spectrum of severity. However, patients with severe AD are the most eligible for systemic/biologic therapies and may therefore be the most appropriate group for using endotypes to target specific therapies in future trials and daily practice. A strength of our study was the confirmation of 3 of the 4 previously identified clusters within a cohort that was not originally designed as a validation cohort.

Although we aimed to divide patients with AD into distinct endotypes based on blood molecular profiles, previous studies have proposed integrating models of lesional and nonlesional skin with blood measures, as well as with clinical parameters, to reflect disease outcomes in AD. Wen et al[35] demonstrated a T_H2/T_H22 profile in blood of Asian patients with AD who had lower T_H1-related cytokine levels than in European American patients, which was attributed to reciprocal downregulation of this axis by the increased T_H17 pathway in the skin.[36] Zhou et al[6] compared AD endotypes among different age groups by evaluating lesional and nonlesional skin, as well as serum biomarkers. This study found decreases in T_H2/T_H22 activation and increases in T_H1/T_H17 axes with age in patients with AD, combined with a normalization of epithelial abnormalities. Although, integrated blood-skin biomarker models might be a more holistic way to build a disease profile, we believe that only serum biomarker endotyping can advance personalized therapeutics and may be more patient friendly. Establishing blood biomarker profiles is particularly crucial in children, in whom skin biopsy specimens are challenging. To confirm our findings in different clinical subgroups of patients with AD, it would be very interesting to perform a separate evaluation of endotypes in a cohort of Asian and/or African American and pediatric and/or elderly patients with AD. Furthermore, biomarker-based cluster analysis in patients with other inflammatory skin diseases, including psoriasis, lichen planus, or contact dermatitis, might be useful as a control for our results in future.

The present study has provided the first step in the confirmation of our previously reported serum biomarker–based patient clusters[9] by replicating 3 of the 4 clusters in a different retrospective cohort. Additionally, we have constructed a prediction model that was able to stratify patients into 1 of the 4 clusters by using only 10 serum biomarkers. The use of a small set of biomarkers to predict patients' cluster status may be easily incorporated into clinical trials and standard practice. Given the introduction of new targeted therapies for AD, the use of endotypes may be helpful because patients with different endotypes might respond differently to the same treatment. Future longitudinal clinical studies are needed to investigate whether the defined endotypes are stable over time and whether patients might switch between clusters over their clinical course (after treatment with systemic immunosuppressive or immunomodulating drugs for instance). Subsequently, confirmation of the endotypes and prediction models in clinical trials that include patients with AD treated with drugs targeting specific pathways will be the final step in confirmation of endotypes in AD.

Key messages

- **In a different population of patients with severe AD, we confirmed the biologic heterogeneity of AD by once more identifying 4 patient clusters based on distinct serum biomarker profiles, 3 of which resembled the previously identified patient clusters. A skin-homing chemokines/IL-1R1–dominant cluster, a $T_H1/T_H2/T_H17$-dominant cluster, a T_H2/T_H22/PARC-dominant cluster, and a T_H2/eosinophil-inferior cluster were identified.**
- **We additionally developed a prediction model based on a small set of biomarkers that were able to classify patients into the 4 clusters.**
- **Stratification of patients with AD into distinct biomarker-based endotypes might contribute to more personalized medicine and may be important to better inform which patients are most likely to benefit from specific targeted therapies.**

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