Letter to the Editor

A computational model to predict severity of atopic eczema from 30 serum proteins

To the Editor:

Evidence-based medicine is more and more required for therapeutic decision making. In the case of atopic eczema (AE), therapeutic effects are measured by clinical observation scores such as the severity scoring of atopic dermatitis (SCORing atopic dermatitis [SCORAD]). These scores are subjective and greatly depend on the investigator, but they remain the best end points because objective biomarkers reflecting therapeutic effects are not available. Nevertheless, during the last decade, several studies aiming at the identification of disease biomarkers in human serum have been performed. This approach seems useful for several reasons: serum is easily accessible and represents a current state of the disease and biomarkers can be used to monitor the efficacy of therapeutic regimens more objectively than with the SCORAD.

Despite the remarkable efforts, until now a single reliable biomarker has not been identified in serum that reflects the severity of AE. Regarding the complexity of disease pathogenesis and high interindividual differences in affected patients, this may

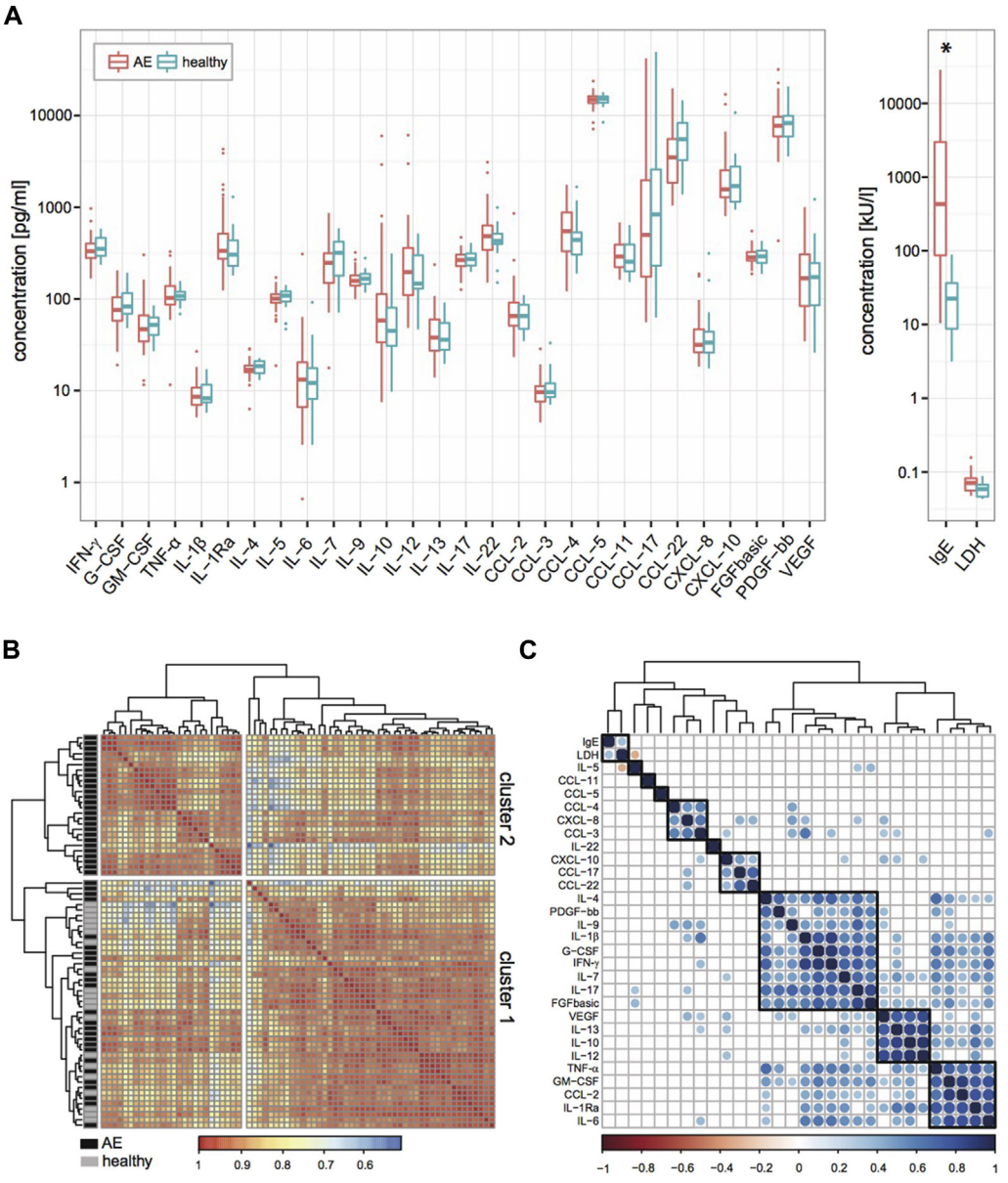
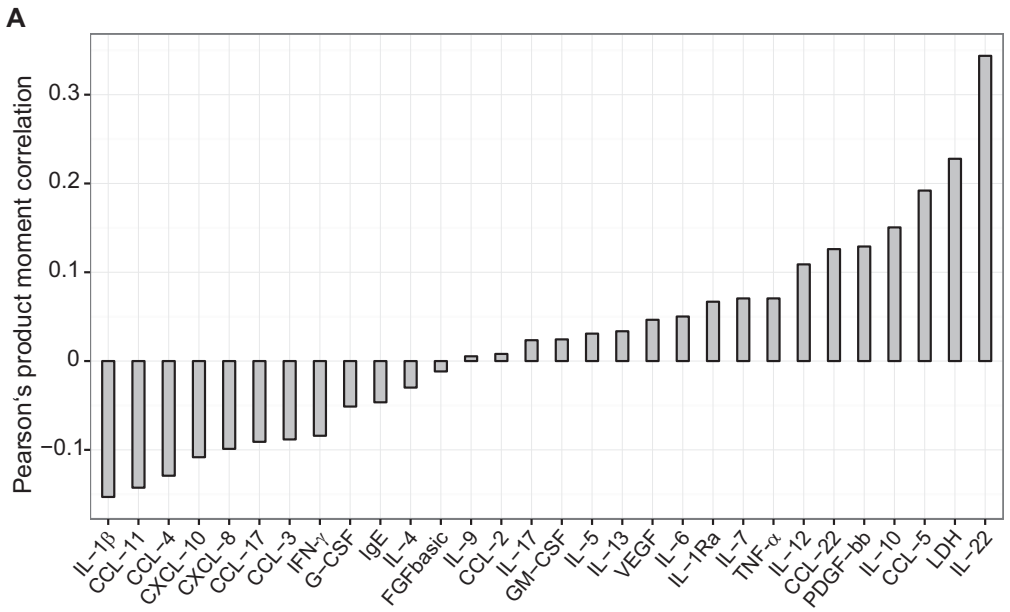
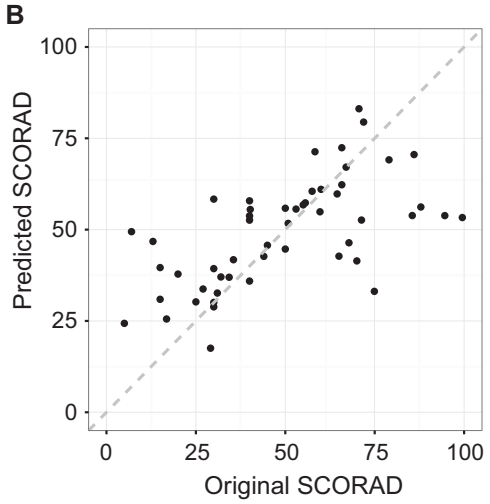


FIG 1. A, Boxplots of 30 serum protein concentrations in log scale of patients with AE (n = 52) and controls (n = 20). *$P < .1$. **B,** Hierarchical clustering of correlation between patients with AE *(black)* and controls *(gray)* indicating the presence of 2 main clusters that substratify patients with AE. **C,** Hierarchical clustering of measured serum proteins in the cohort with AE. $(1 - r)$ was used as distance measure and the ward.D2 clustering method was used for identifying clusters. In total, 6 protein clusters could be defined.





	Estimate	Standard Error of Estimate	T Statistic	(two-sided) p value
(Intercept)	-399.4726	172.4621	-2.3163	0.0259
IL-1ß	-58.4658	37.9438	-1.5409	0.1314
CXCL-8	-29.2403	19.1570	-1.5263	0.1350
IL-12	36.8274	16.7418	2.1997	0.0338
IL-13	-81.6104	34.3172	-2.3781	0.0224
CXCL-10	-19.5963	12.4115	-1.5789	0.1224
CCL-2	-15.7470	15.4502	-1.0192	0.3144
CCL-3	78.4862	39.2006	2.0022	0.0523
CCL-5	48.5214	36.6568	1.3237	0.1933
TNF-α	78.7947	29.3231	2.6871	0.0105
CCL-22	23.9886	11.8338	2.0271	0.0495
IL-22	25.9447	13.4064	1.9353	0.0602
LDH	50.8819	29.6462	1.7163	0.0940

FIG 2. A, Serum proteins were correlated with the SCORAD using the Pearson's product-moment correlation. No significant correlation was observed. **B,** SCORAD prediction model. The best-fit model identified 12 serum proteins (shown in the table) for optimal SCORAD prediction (graph on the *left*). The table gives information on the 12 parameters (serum proteins) included and the intercept, estimate, and *P* value. The *P* value tests whether the estimated coefficient in the model is significantly different from zero. The adjusted R^2 for the best-fit model is 0.198, with a leave-one-out cross-validation root mean squared error of 22.8.

not be surprising. We therefore aimed to investigate the potency of a biomarker signature, a combination of serum proteins rather than a single biomarker, to model and predict the severity of AE. For this purpose, serum of 52 patients with AE diagnosed after the criteria of Hanifin and Rajka and histologic evaluation (29 men, 23 women; age, 37.8 ± 20.1 years; SCORAD, 49.1 ± 23.5; total IgE, 2355 ± 4575 kU/L [values represent mean and SD]) and 20 healthy controls with no history of AE and total IgE level of 29.1 ± 26.4 kU/L (8 men, 12 women; age, 37.8 ± 10.0 years) was analyzed for the presence of 32 serum proteins using the Bio-Plex Pro Human Cytokine 27-plex Assay (Bio-Rad, Munich, Germany) (for composition, see Table E1 in this article's Online Repository at www.jacionline.org), and single plexes for CCL17 and CCL22 (Bio-Rad) as well as IL-22 (ELISA; R&D Systems, Minneapolis, Minn) and lactatedehydrogenase (LDH; Abcam, Cambridge, United Kingdom) and total IgE (ImmunoCap; Phadia, Freiburg, Germany). The quantitative composition of all measured proteins is shown in Fig 1, *A*. The severity of AE was determined in all patients using SCORAD, which evaluates the intensity, extent, and subjective signs of the disease. Analysis of $\log_{10}$-transformed parameter values and SCORAD prediction was conducted in R.[1] All R codes used for statistical analysis can be provided on request.

Two proteins (IL-2 and IL-15) were not detectable in serum of more than 25% of the patients and controls and were therefore excluded from subsequent analysis. Besides IgE, significant

differences between serum protein concentrations of patients and controls could not be observed when applying a Welch 2-sample *t* test with a false-discovery rate (FDR) of 10% (Fig 1, *A*). This is in line with published reports and supposedly due to the high interindividual differences in the patient and control groups.[2] When performing a hierarchical clustering based on the Pearson product-moment correlation between probands, 2 clusters can be identified with one containing patients and controls (cluster 1) and one with patients only (cluster 2) (Fig 1, *B*). Significant differences between the 2 clusters were detected for IgE and LDH (see Fig E1 in this article's Online Repository at www.jacionline.org), but not for the other parameters investigated (10% FDR). Here, IgE and LDH concentrations are higher in cluster 2 and indicate the separation of an extrinsic eczema subgroup from an intrinsic eczema subgroup.

To get a first glimpse on potential interparameter relations, a pairwise correlation analysis and subsequent hierarchical clustering was performed. We used $(1 - r)$ as a distance measure and the "ward.D2"[3] clustering method to identify clusters (Fig 1, *C*). In total, 6 sets of proteins containing at least 2 proteins were detected in the patient cohort, hinting at protein combinations that potentially are linked together in pathogenesis.

Even if no significant differences exist between patients and controls, single serum proteins might correlate with the SCORAD in patients. However, based on the Pearson's product-moment correlation, no significant correlations between single proteins and SCORAD were detected (based on FDR < 10%) (Fig 2, *A*; Table E1). Interestingly, none of the T_H2-associated cytokines such as IL-4 and IL-5, which represent the hallmarks of immunological deviation in AE, showed either difference between patients and controls or correlation with the SCORAD in this and other studies.[2,4] A reason for this might be the biphasic course of atopic eczema, being dominated by T_H2 cytokines in the acute phase and T_H1 cytokines in the chronic phase.[5] Hence, these cytokines may have functional relevance in disease pathology, but they are not suitable as biomarkers for AE. In addition, CCL17, CCL22, and LDH have been postulated as biomarkers for AE severity.[6] However, in our cohort, none of these markers significantly correlated with the SCORAD. This is in line with observations from other groups that reported high interindividual differences in serum concentrations of these proteins.[6]

Because single proteins were not suitable indicators of AE severity, a statistical model was used that selects protein combinations to predict the SCORAD. The SCORAD outcome was learned using a partial least squares linear regression model with $\log_{10}$-transformed protein concentrations as covariates. On performing all (parameter) subset regression analysis with the regsubsets function from the leaps[7] package in R and optimizing the adjusted residual sum of squares (R^2), we found that the identified optimal model included 12 serum proteins (Fig 2, *B*, table). This SCORAD predictive model is a weighted sum of the intercept that represents the baseline SCORAD and the slope that is calculated using the $\log_{10}$ transformed concentration of the 12 serum proteins multiplied by their respective estimated coefficient. The adjusted R^2 is a criterion for the quality of the model—the closer to 1, the better the model. In the established model, the adjusted R^2 was very low with 0.198 and the root mean squared prediction error of leave-one-out cross-validation being 22.8 (Fig 2, *B*). In comparison, the adjusted R^2 for the model including all measured proteins (n = 30) was −0.298 and the result of leave-one-out cross-validation was 39.7. So, even the optimal fitted combination of 12 proteins left us with a prediction error of 23 SCORAD points. Even if SCORAD is a subjective tool critically depending on the investigator, the precise clinical description seems superior to this prediction model.

Taken together, no significant correlation of 1 of the 30 serum proteins investigated with the SCORAD was discovered. In addition, even the establishment of a SCORAD prediction model using the best-fit combination of proteins delivered an error value that is not acceptable for indicating therapeutic SCORAD changes. With the given techniques and markers, even state-of-the-art bioinformatics cannot construct a reliable and objective prediction tool to measure therapeutic effects in AE from serum.

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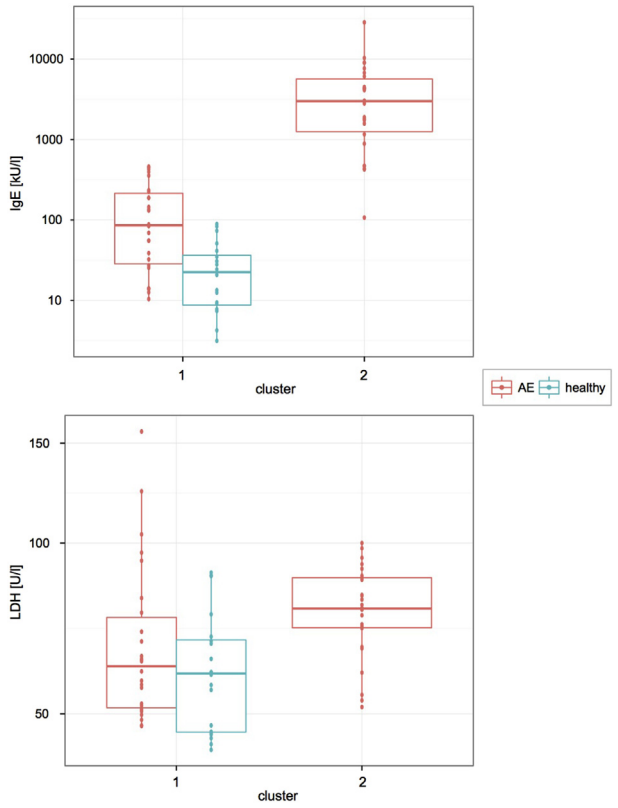

FIG E1. Significant differences between the hierarchical clusters 1 and 2.

TABLE E1. Pearson correlation coefficients of all serum proteins and SCORAD

Serum proteins	Pearson correlation coefficient	*P* value	Adjusted *P* value
IL-22	0.34374	.01260	.37787
LDH	0.22780	.10434	.96522
CCL-5	0.19201	.17268	.96522
IL-10	0.15052	.28683	.96522
PDGF-bb	0.12900	.36206	.96522
CCL-22	0.12606	.37319	.96522
IL-12	0.10889	.44222	.96522
TNF-α	0.07057	.61907	.96522
IL-7	0.07054	.61924	.96522
IL-1Ra	0.06683	.63786	.96522
IL-6	0.05018	.72390	.96522
VEGF	0.04646	.74360	.96522
IL-13	0.03358	.81319	.96522
IL-5	0.03089	.82791	.96522
GM-CSF	0.02442	.86358	.96522
IL-17	0.02349	.86870	.96522
CCL-2	0.00803	.95495	.96986
FGFbasic	0.00537	.96986	.96986
IL-9	−0.01168	.93451	.96986
IL-4	−0.02987	.83353	.96522
IgE	−0.04644	.74372	.96522
G-CSF	−0.05123	.71834	.96522
IFN-γ	−0.08414	.55316	.96522
CCL-3	−0.08826	.53382	.96522
CCL-17	−0.09096	.52131	.96522
CXCL-8	−0.09889	.48550	.96522
CXCL-10	−0.10829	.44477	.96522
CCL-4	−0.12913	.36158	.96522
CCL-11	−0.14262	.31315	.96522
IL-1β	−0.15305	.27871	.96522

CCL, CC chemokine ligand; *CXCL*, CXC chemokine ligand; *FGF*, fibroblast growth factor; *LDH*, lactate dehydrogenase; *PDGF*, platelet derived growth factor; *TNF*, tumor necrosis factor; *VEGF*, vascular endothelial growth factor.