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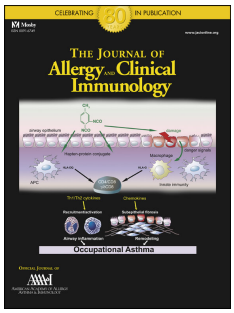

A computational model to predict severity of atopic eczema from 30 serum proteins

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

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Capsule summary:
Prediction of atopic eczema severity by use of serum proteins is a helpful tool for monitoring objective therapeutic response. Here, we applied advanced computational models to identify the optimal combination out of 30 serum proteins for SCORAD prediction in a large patient series.

Key words:
Atopic eczema, SCORAD prediction, serum, biomarker

Abbreviations:
AE = atopic eczema; SCORAD = SCORing atopic dermatitis; est. = estimated coefficient; r = Pearson's product moment correlation; FDR = false discovery rate; R^2 = residual sum of squares

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Conflict of interest:
The authors state no conflict of interest

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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 (SCORAD). These scores are subjective and greatly depend on the investigator, but they remain the best endpoints as 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 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 inter-individual differences of affected patients, this may 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 AE patients diagnosed after the criteria of Hanifin and Rajka and histologic evaluation (29 male, 23 female, age 37.8 ± 20.1 years, SCORAD 49.1 ± 23.5; total IgE 2355 ± 4575 kU/l (values represent mean and standard deviation)) and 20 healthy controls with no history of AE and total IgE <100 kU/l (8 male, 12 female, age 37.8 ±10.0 years) was analyzed for presence of 32 serum proteins using the Bio-Plex Pro™ Human Cytokine 27-plex Assay (Biorad) (see Table E1 for composition), and single plexes for CCL17 and CCL22 (Biorad) as well as IL-22 (ELISA, R&D) and Lactatedehydrogenase (LDH, Abcam) and total IgE (ImmunoCap). The quantitative composition of all measured proteins is shown in Fig. 1A. The severity of atopic eczema was determined in all patients using the severity scoring of atopic dermatitis (SCORAD) that evaluates intensity, extent and subjective signs of the disease. Analysis of log10 transformed parameter values and SCORAD prediction was conducted in R[1]. All R codes used for statistical analysis can be provided upon request.

Two proteins (IL-2 and IL-15) were not detectable in serum of more than 25% of 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 two sample t-test with a false discovery rate (FDR) of 10% (Fig.1A). This is in line with published reports and supposedly due to the high inter-individual differences in the patient and control group[2]. When performing a hierarchical clustering based on the Pearson product moment correlation between probands, two clusters can be identified with one containing patients and controls (cluster 1) and one with patients only (cluster 2) (Fig.1B). Significant differences between the two clusters were detected for IgE and LDH (Fig.E1), 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 from an intrinsic eczema subgroup.

To get a first glimpse on potential inter-parameter relations, a pair-wise 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.1C). In total, six sets of proteins containing at least two 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.2A and Table E1). Interestingly, none of the Th2 associated cytokines such as IL-4 and IL-5 that represent the hallmarks of immunological deviation in AE neither showed difference between patients and controls, nor were correlated 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 Th2 cytokines in the acute phase and Th1 cytokines in the chronic phase[5]. Hence these cytokines may have functional relevance in disease pathology, 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 inter-individual differences in serum concentrations of these proteins[6].

As 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 log10 transformed protein concentrations as covariates. Performing all (parameter) subset regression analysis with the regsubsets function from the leaps[7] package in R and optimizing the adjusted R^2, the identified optimal model included twelve serum proteins (Fig.2B 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 twelve serum protein concentrations multiplied by their respective estimated coefficient. The adjusted R^2 is a criterion for the quality of the model – the closer to one 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.2B). 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 39.7. So, even the optimal fitted combination of twelve 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 one 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 can´t construct a reliable and objective prediction tool to measure therapeutic effects in AE from serum.

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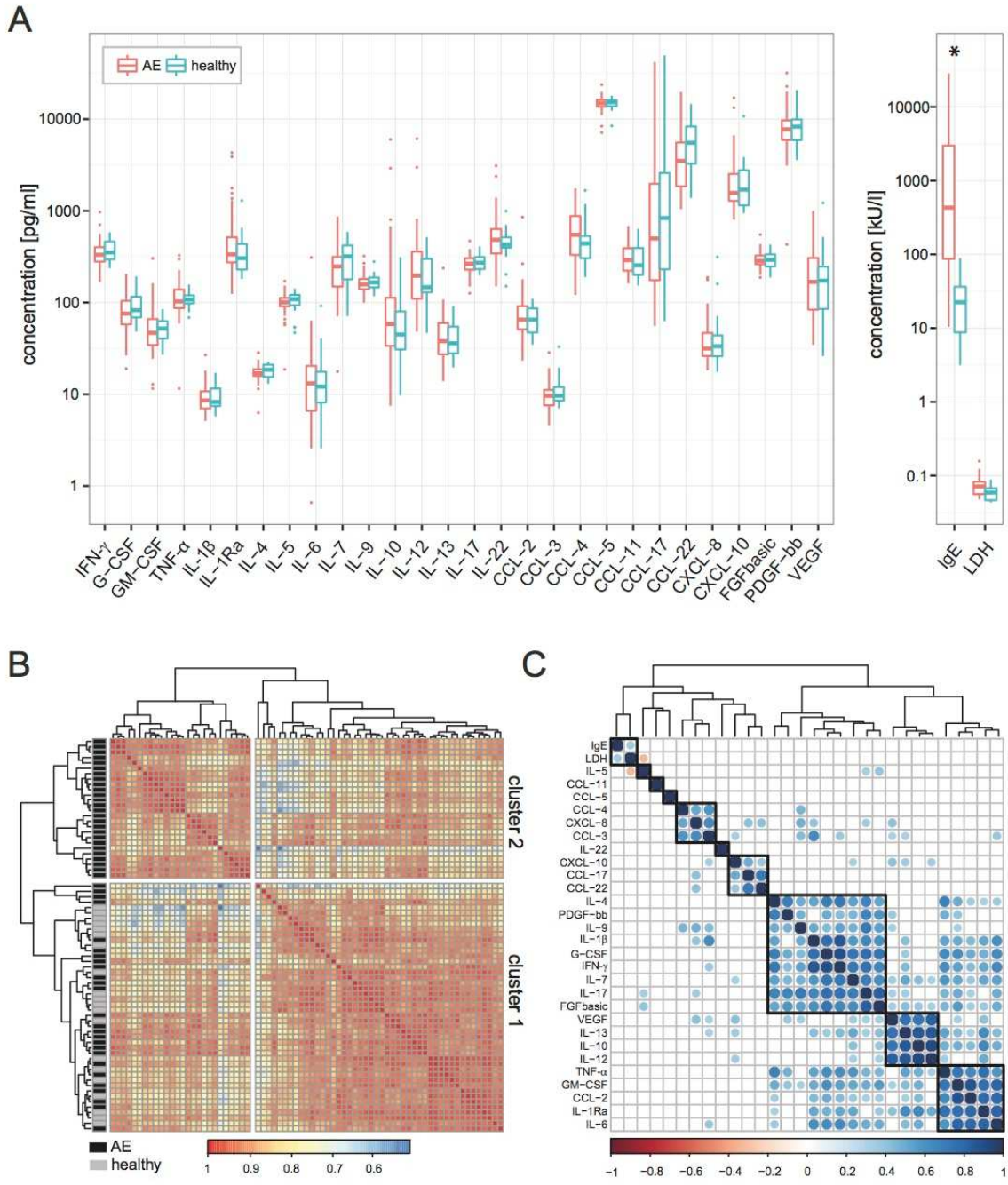
Figures and Tables

Figure 1:
A) Boxplots of 30 serum protein concentrations in log scale of AE patients (n=52) and controls (n=20). *indicates significance with p<0.1. B) Hierarchical clustering of correlation between AE patients (black) and controls (grey) indicating the presence of two main clusters that sub-stratify AE patients. C) Hierarchical clustering of measured serum proteins in the AE patient cohort. (1-r) was used as distance measure and the ward.D2 clustering method for identifying clusters. In total, six proteins clusters could be defined.

Figure 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 twelve serum proteins (shown in the table) for optimal SCORAD prediction (graph on the left). The table gives information on the twelve 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.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IL-13
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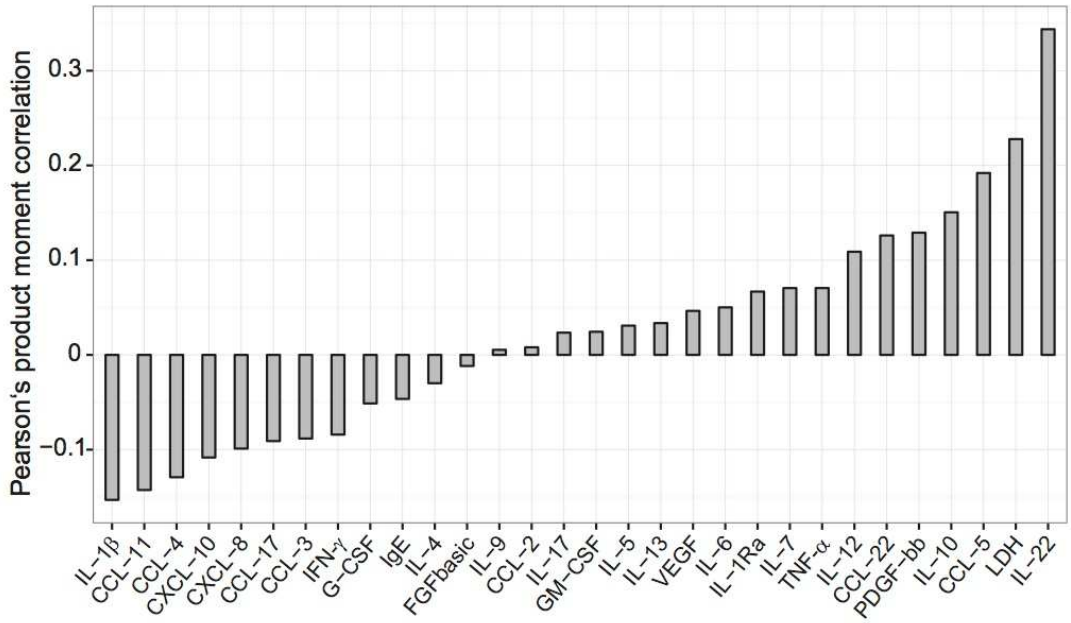

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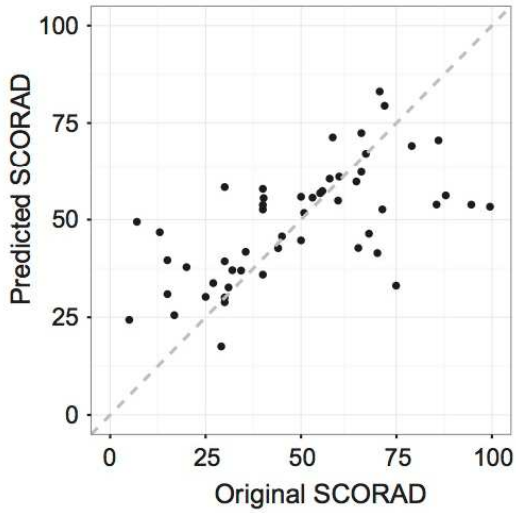


	Estimate	Standard Error of Estimate	T Statistic	(two-sided) p value
(Intercept)	-399.4726	172.4621	-2.3163	0.0259
`IL-1b`	-58.4658	37.9438	-1.5409	0.1314
`CXCL-8`	-29.2403	19.1570	-1.5263	0.1350
`IL-12`	36.8274	16.7418	2.1997	0.0338
`IL-13`	-81.6104	34.3172	-2.3781	0.0224
`CXCL-10`	-19.5963	12.4115	-1.5789	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-a`	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

Supplemental figures and tables

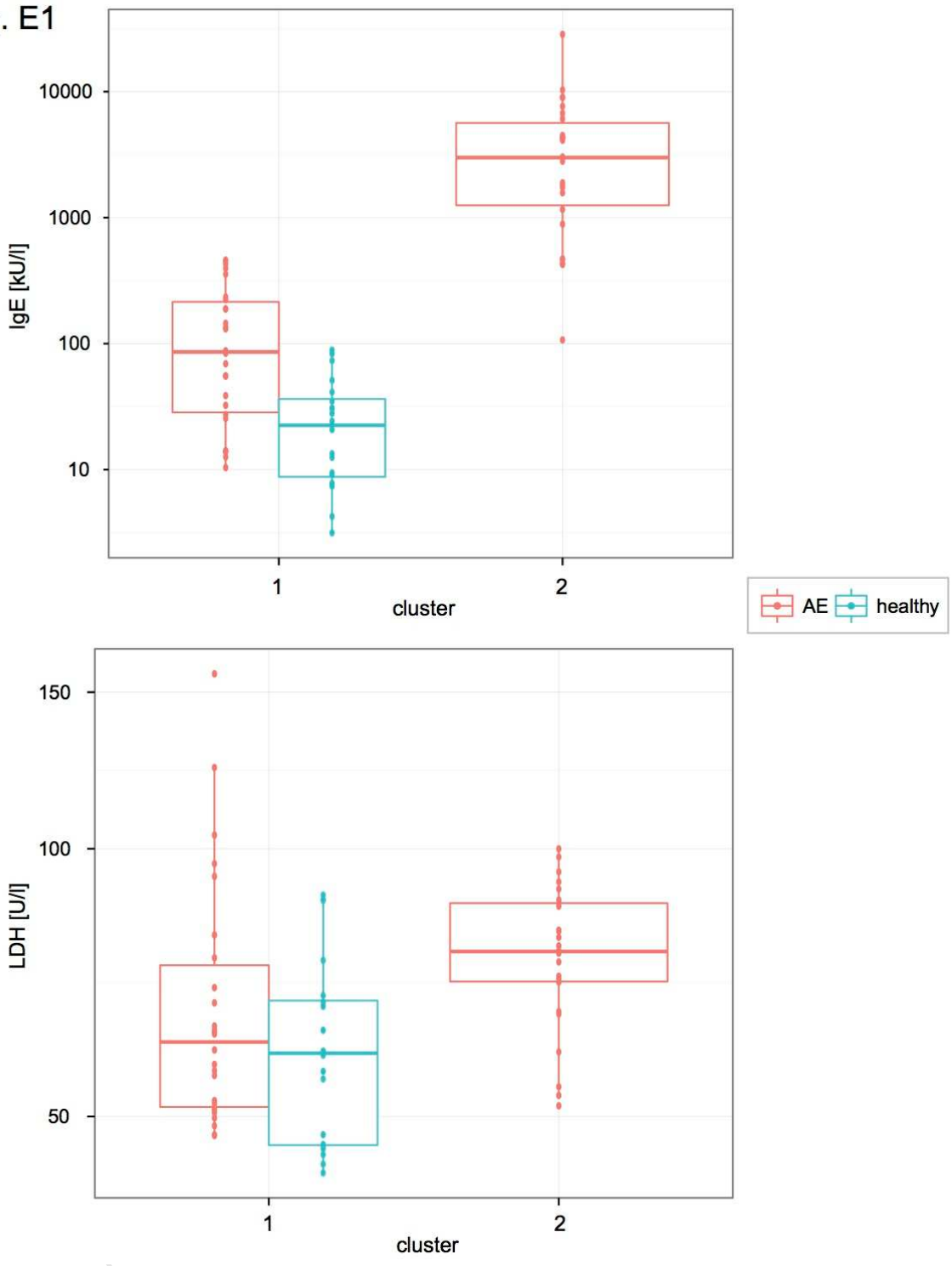
Figure E1: Significant differences between the hierarchical clusters 1 and 2.

Table E1: Pearson correlation coefficients of all serum proteins and SCORAD

	pearson correlation coefficient	p-value	adjusted p-value
IL-22	0.34374	0.01260	0.37787
LDH	0.22780	0.10434	0.96522
CCL-5	0.19201	0.17268	0.96522
IL-10	0.15052	0.28683	0.96522
PDGF-bb	0.12900	0.36206	0.96522
CCL-22	0.12606	0.37319	0.96522
IL-12	0.10889	0.44222	0.96522
TNF-α	0.07057	0.61907	0.96522
IL-7	0.07054	0.61924	0.96522
IL-1Ra	0.06683	0.63786	0.96522
IL-6	0.05018	0.72390	0.96522
VEGF	0.04646	0.74360	0.96522
IL-13	0.03358	0.81319	0.96522
IL-5	0.03089	0.82791	0.96522
GM-CSF	0.02442	0.86358	0.96522
IL-17	0.02349	0.86870	0.96522
CCL-2	0.00803	0.95495	0.96986
FGFbasic	0.00537	0.96986	0.96986
IL-9	-0.01168	0.93451	0.96986
IL-4	-0.02987	0.83353	0.96522
IgE	-0.04644	0.74372	0.96522
G-CSF	-0.05123	0.71834	0.96522
IFN-γ	-0.08414	0.55316	0.96522
CCL-3	-0.08826	0.53382	0.96522
CCL-17	-0.09096	0.52131	0.96522
CXCL-8	-0.09889	0.48550	0.96522
CXCL-10	-0.10829	0.44477	0.96522
CCL-4	-0.12913	0.36158	0.96522
CCL-11	-0.14262	0.31315	0.96522
IL-1β	-0.15305	0.27871	0.96522

Table E1: Pearson correlation coefficients of all serum proteins and SCORAD

Fig. E1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