Methods

Patients

We included 160 consecutive, newly referred outpatients with AD older than 4 years of age (who met the criteria defined by Hannifin and Rajka [11]) from the Department of Dermatology, Bispebjerg Hospital, University of Copenhagen, Denmark, between January 2012 and June 2018 [10].

Assessments

AD diagnosis according to criteria was ensured by a senior physician. Severity was assessed by a trained staff nurse using SCORAD, which is a commonly used severity index for AD (range 0--103 points with a high score indicating severe disease) [12]. Patients were divided into disease severity groups based on SCORAD into mild (SCORAD <25), moderate (SCORAD range 25--50), and severe disease (SCORAD >50) [13–15]. Patients were divided into children/adolescents (≤15 years of age) and adults (>15 years of age) [10].

Serum samples were obtained by a staff biotechnician and immediately stored at --18°C. Samples were subsequently stored in a freezer at --80°C. Prior to analysis, all samples were transported on dry ice to the Academic Medical Center, University of Amsterdam, The Netherlands. Cytokine concentrations were measured using the MESO QuickPlex SQ 120 (MSD, Rockville, MA, USA) according to the manufacturer’s instructions, apart from the samples being undiluted and incubation time prolonged to 16 h. Cytokines included were interleukins (ILs) 1β, 2, 4, 5, 8, 10, 16, 17E/25 (IL-25), 17A, 22, 31, and 33, and CCL2 (also termed monocyte chemotactic protein-1 [MCP-1]), CCL11 (also termed eotaxin), CCL17 (chemokine-ligand-17, also termed thymus and activation-regulated chemokine [TARC]), CCL22 (also termed macrophage-derived chemokine [MDC]), CCL26 (also termed eotaxin-3), CCL27 (also termed cutaneous T cell-attracting chemokine [CTACK]), and TSLP (thymic stromal lymphopoietin). The chemical nomenclature is used from now on. All biomarkers were reported in pg/mL and were selected based on previous studies [3, 16]. Biomarker values below the fit curve range were given half the value of the lowest measured value on that plate (e.g., CCL11). A single CCL22 sample had a value above the fit curve range and was arbitrarily assigned twice the highest value detected.

All patients filled in a questionnaire using questions implemented from the ISAAC (International Study of Asthma and Allergies in Childhood) and ECRHS (European Community Respiratory Health Survey) studies [17, 18]. The questionnaire explored demographics (sex and age), comorbidities (asthma, allergic rhinoconjunctivitis, food allergy, and urticaria) and AD characteristics (age of onset, a family history of AD, and eczema distribution within the past month). Assistance with filling in the questionnaire was allowed for patients ≤15 years, but not from a hospital staff member.

Blood samples from all patients was genotyped for the 3 most common variations in *FLG*; R2447X, R501X and 2282del4, and analyzed for serum total IgE levels (103 IU/L) and blood eosinophil count (cell count × 109/L). Serum total IgE levels digitally truncated as >2,000 or >2,500 × 103 IU/L were denoted as 2,000 and 2,500 × 103 IU/L, respectively.

Patients with high serum total IgE (≥150 × 103 IU/L) and/or a positive allergen test (skin prick test and/or RAST-test [>0.35 kU/L]) specific for ≥1 allergens from the standard inhalation panel (birch, grass, mugwort, horse, dog, cat, house dust mite [*D. pteronyssinus* and *D. farinae*] and moulds [*A. iridis* and *C. herbarium*]) were grouped as extrinsic AD. Patients with normal serum total IgE (<150 × 103 IU/L) and a negative allergen test to the standard panel (skin prick test and/or RAST test [<0.35 kU/L]) was grouped as intrinsic AD.

Statistical analyses

Spearman’s correlation analysis was used to compare individual serum biomarker levels with disease severity (SCORAD), using IBM SPSS statistics v22 (SPSS, Inc., Chicago, IL, USA). Multiple comparisons adjustment was performed using Bonferroni correction.

Independent samples *t* test and χ2 tests were performed independently using children/adolescents vs. adults, *FLG* variation carrier status, extrinsic/intrinsic AD subtype, and asthma as response variables, and biomarker levels, sex, age, allergic rhinoconjunctivitis, food allergy, urticaria, age at onset of AD, serum total IgE, and blood eosinophil count as explanatory variables.

We developed 2 predictive models for SCORAD from 22 biomarkers (19 cytokines, *FLG* variation, serum total IgE, and blood eosinophil count) and the patient’s history of atopy (asthma, allergic rhinoconjunctivitis, food allergy, or urticaria), age, and sex, using a linear regression with elastic net (ENET) regularization [19] and gradient boosting machine, respectively. Hyperparameter tuning and performance evaluation were conducted in a nested *k*-fold cross-validation (*k* = 10). The predictive performance was assessed with the root mean square error (RMSE) and MCID accuracy (the proportion of prediction within 1 MCID of the true score [20]). Calculations were performed using *R* v3.5.1 (*R* Foundation for Statistical Computing, Vienna, Austria).