

Correspondence

Multiplex platform technology and bioinformatics are essential for development of biomarkers in atopic dermatitis



To the Editor:

In the recently published letter to the Editor by Krause et al,¹ the authors tried to establish a combination of serum proteins that could predict disease severity in patients with atopic dermatitis (AD). Serum levels of 32 protein markers were determined in 52 patients with AD and 20 healthy controls. To our surprise, the authors found no significant differences between the levels of 31 serum proteins in patients and controls, with the exception of total IgE levels. This is highly remarkable because many previous publications have reported increased levels of these serum biomarkers, including IL-4, macrophage-derived chemokine, and thymus and activation-regulated chemokine (TARC/CCL17) in patients with AD compared with healthy controls.²⁻⁴ Krause et al found serum TARC levels in healthy controls ranging from around 90 pg/mL to more than 20,000 pg/mL. This is remarkable because from literature and our own experience we know that serum TARC levels in healthy controls do not exceed 600 pg/mL.^{2,5-7}

Krause et al also investigated correlations between serum biomarker levels and disease severity measured by SCORing Atopic Dermatitis. Surprisingly, they found no significant correlations between biomarker levels and SCORing Atopic Dermatitis. This also is in contrast to several previous publications, which are summarized in a recently published meta-analysis.⁸ In this meta-analysis, we show that serum TARC levels have been determined in a relatively large number of patients from different studies, showing pooled correlation coefficients of 0.60 (95% CI, 0.48-0.70) and 0.64 (95% CI, 0.57-0.70) in longitudinal and cross-sectional studies, respectively.⁹

The authors conclude that their findings are in line with our publication. We indeed reported that TARC levels vary between patients within cross-sectional cohorts of patients who have similar disease severity scores; however, the overall correlation that we found between serum TARC levels and disease severity is still strong.^{5,10} Considering the interindividual differences in serum TARC levels and the heterogeneous character of AD, we do agree with the authors on the necessity of a combination of biomarkers for measuring disease severity in AD.

Therefore, we recently conducted a pilot study in which we demonstrated that a multivariate signature, consisting of serum TARC, PARC/CCL18, IL-22, and sIL-2R levels, showed a correlation coefficient of 0.86 to disease severity.¹¹

We strongly believe that state-of-the-art multiplex platform technology and bioinformatics can help in developing reliable and objective prediction tools to measure therapeutic effects in AD from serum.

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Reply



To the Editor:

We thank Thijs et al¹ for the helpful comments on our article.² Indeed, this correspondence led to the identification of misassignments of healthy controls and atopic eczema. We therefore re-performed our analysis, which led to the identification of significantly different serum levels for CCL17, CCL22, CXCL10, IgE, and lactate dehydrogenase between healthy controls and patients with atopic eczema. CCL17 levels in the healthy control group are now lower, yet still range from 56 pg/mL to 979 pg/mL, with an average of 169 ± 202 pg/mL (mean $\pm$ SD) in our cohort. Despite high interindividual variations in the CCL17 expression levels in patients with atopic eczema (4629 ± 9559 pg/mL), we can confirm an overall correlation of CCL17 with the SCORing Atopic Dermatitis (SCORAD)

(Pearson correlation coefficient of log10-transformed CCL17 is 0.450; adjusted *P* value = .00084; 95% CI, 0.201-0.643) in our data set. This correlation is in the lower range when compared with the cross-sectional studies of the meta-analysis by Thijs et al.³ This further hints at large disease variability, which is in line with the broad acceptance in the field and underpins the need of a multimarker biomarker to predict the severity of atopic eczema rather than a single biomarker. Similar to the multimarker disease severity model developed by Thijs et al.,⁴ predictions of the SCORAD using our multimarker model are correlated with the original/clinical SCORAD values at 0.759 (95% CI, 0.612-0.855). However, computing the correlation coefficient between predicted and true values cannot assess the goodness of the fit of any predictive model. This is best demonstrated using our SCORAD model: despite the good overall correlation, the mean squared prediction error estimated by leave-one-out cross-validation pinpoints a prediction error of 18.9 SCORAD points at patient level. Thus, even this optimized best-fit prediction model underestimates or overestimates the severity of atopic eczema by 20%. We, therefore, cannot compare our findings to the model of Thijs et al, which was not evaluated by any model-based quality measure. Overall, we agree with the conclusions of Thijs et al that the combination of biomarkers and usage of bioinformatics tools may be the way to develop more reliable and stable tools to monitor disease severity; however, arguing from our data, we still may lack the perfect markers to be included into these sophisticated models. Currently, there is an unmet medical need to predict disease severity of atopic eczema at individual patient's level.

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Comparing sensitivity of Hymenoptera allergen components on different diagnostic assay systems: Comparing apples and oranges?



To the Editor:

In a recent letter to the editor published in the *Journal*, Schrautzer et al¹ reported the comparison of 2 different diagnostic assays, namely ImmunoCAP (Thermo Fisher Scientific, Waltham, Mass) and Immulite (Siemens, Tarrytown, NY), for the detection of specific IgE (sIgE) responses to allergen components in a population of patients with Hymenoptera venom allergy. The study reports a higher rate of positive test results (≥ 0.35 kU_A/L) on the Immulite compared with the ImmunoCAP system for sIgE to rApi m 1 in patients with bee venom allergy and for sIgE to rVes v 5 in patients with vespid venom allergy and concludes that the Immulite system displays a higher sensitivity for the detection of sIgE to these Hymenoptera venom allergens. Similar observations were reported in another recent publication by Selb et al.² In particular, the sensitivity of rApi m 1 has been a matter of debate.²⁻⁴ Schrautzer et al¹ suggest that the rApi m 1 used in the ImmunoCAP has a reduced IgE-binding capacity and speculate that this might be due to altered protein folding without reference to a previous report in the same journal that had demonstrated equivalent IgE antibody binding of this particular recombinant protein and purified natural Api m 1.⁴

A test system with increased sensitivity and comparable specificity would certainly be of benefit for the diagnosis and management of patients with Hymenoptera venom allergy; however, a few issues need to be raised that have not adequately been considered by Schrautzer et al.¹ Systematic differences in assay characteristics of the 2 test systems have been reported before.⁵⁻⁷ Both systems use a calibration concept based on total IgE (tIgE) for determination of sIgE levels. Mouse-human chimeric IgE antibodies with defined allergen monospecificity and concentration have been used to evaluate the accuracy of sIgE measurements in different assay systems.⁵⁻⁷ In this approach IgE antibody of a single specificity comprises the entire amount of IgE present in the sample, allowing assessment of the system's calibration for sIgE by comparing the sIgE and tIgE measurements it delivers. Although equivalent sIgE and tIgE results were obtained with the ImmunoCAP system, the Immulite system reported sIgE concentrations that were on average 2- to 3-fold higher than the tIgE concentrations in the same sample. Considering that the tIgE measurements agreed well between systems and with the predefined tIgE concentration of the samples, the findings demonstrated that Immulite delivers overestimated sIgE levels. This will inevitably lead to a greater number of test results exceeding any given cutoff value but does not represent a higher sensitivity of the assay. Because a similar sIgE/tIgE discordance has already been reported for 5 different allergens (Bet v 1, Can f 1, Der p 2, Fel d 1, and Gal d 1),⁵⁻⁷ it is likely to apply also to the Hymenoptera venom allergens discussed here and explain the seemingly more sensitive detection of sIgE to rApi m 1 and rVes v 5 reported by Schrautzer et al.¹ In line with this is the observation by Selb et al² that positive values of rApi m 1 and rVes v 5 were, on average, 2.7 and 2.3 times higher with the Immulite than with the ImmunoCAP system.² Unfortunately, Schrautzer et al¹ did not provide data on the actual sIgE