

Microarray-based IgE detection in capillary blood samples of patients with atopy

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Key words: allergic rhinitis; asthma; capillary serum; IgE; microarray.

In vitro diagnosis of IgE-mediated sensitization currently relies on fluorescence enzyme immunoassays (FEIA) requiring peripheral venipuncture which can be cumbersome and sometimes painful particularly in infants and young children. However, microarray technology has recently been introduced into allergological research detecting specific IgE antibodies against numerous allergens in as little as 50 µl of patient serum, a probe volume easily obtained by capillary blood sampling (1, 2). Still, the feasibility of microarray-based IgE detection using capillary blood-derived serum specimen has not been evaluated at all so far.

Thus, we prospectively recruited 47 children and adults (23 male, 24 female; median age 22.00 ± 17.08 years) treated for allergic rhinitis and/or extrinsic bronchial asthma in whom sensitization to one or more tree pollen (birch, hazel, alder) or timothy grass pollen allergen extracts had previously been established by conventional FEIA analysis (UniCAPTM; Pharmacia Diagnostics, Uppsala, Sweden). All patients routinely underwent peripheral venous blood withdrawal,

Low volumes of capillary serum are suitable for microarray-based IgE detection in patients with atopy.

additional capillary blood sampling was performed on the fingertip with a blood lancet (FeatherTM; Safety Razor Ltd, Osaka, Japan). This study was approved by the ethics committee of the medical faculty, University of Aachen (EK 052/03).

Following similar processing of whole blood specimen, a volume of 50 µl was derived from venous and capillary serum samples respectively. All probes were screened for allergen-specific IgE applying a customized, commercially available allergen microarray (ISACTM Atopy; VBC-Genomics, Vienna, Austria) containing recombinant birch (rBetv1, rBetv2), alder (rAlng1), hazel (Cora1) and timothy grass pollen (rPhlp1, rPhlp5, rPhlp6 and rPhlp7) components. The corresponding microarray procedures were carried out by the same investigator strictly adhering to assay protocols previously published (3, 4). Finally, levels of allergen-specific IgE antibodies were visualized transforming microarray image information into numerical data quantified as fluorescence intensity (FI) values. Statistical analyses were performed using the S PlusTM 6.1 software

package (Insightful Corp., Seattle, WA) and Pearson's correlation coefficient was calculated with regard to microarray results after venous and capillary blood sampling.

All patients revealed IgE antibodies against at least one recombinant tree pollen or timothy grass pollen component implemented on the allergen microarray. The majority proved to be sensitized against the birch pollen major allergen rBetv1 (76.6% of patients) and timothy grass pollen allergen rPhlp1 (66%) whereas allergen-specific IgE to other components was found less frequently (rBetv2: 48.9%; rPhlp6: 44.6%; rPhlp5: 42.5%; rAlng1: 42.5%; rCora1: 31.9%; rPhlp7: 19.1%). Analyzing each allergen component separately, the according IgE profiles revealed a high or even very high correlation coefficient (*r*) between microarray results after capillary and venous serum sampling: $r_{\text{Betv1}} = 0.93$; $r_{\text{Betv2}} = 0.89$; $r_{\text{Cora1}} = 0.97$; $r_{\text{Alng1}} = 0.98$; $r_{\text{Phlp1}} = 0.94$; $r_{\text{Phlp5}} = 0.97$; $r_{\text{Phlp6}} = 0.97$; $r_{\text{Phlp7}} = 0.98$. In order to illustrate overall comparability, we calculated the correlation coefficient of all acquired venous and

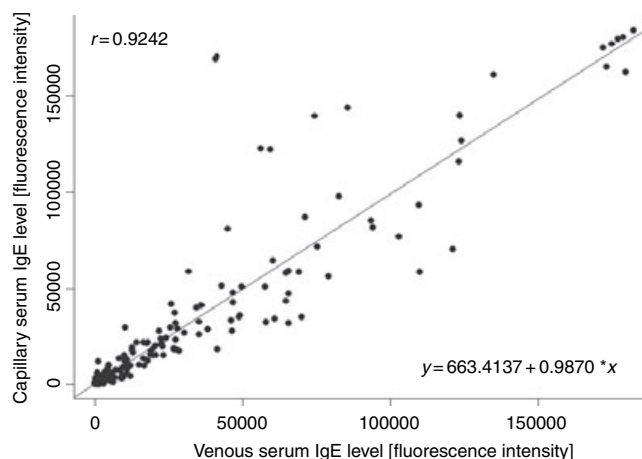


Figure 1. Correlation between venous (*x*) and capillary (*y*) allergen microarray results for the recombinant tree pollen allergen components rBetv1, rBetv2, rCora1, rAlng1 and the timothy grass pollen components rPhlp1, rPhlp5, rPhlp6 and rPhlp7. Results are displayed as a scatter plot of IgE microarray data quantified as fluorescence intensity (FI) values with the according regression line and regression line equation as well as the corresponding Pearson correlation coefficient (*r*).

capillary microarray data as visualized in Fig. 1.

To our knowledge, this is the first investigation focusing on capillary serum sampling for microarray-based IgE analysis. Our data suggest that the detection of specific IgE antibodies against multiple recombinant allergen components is feasible and reliable utilizing only minute volumes of patient serum. Allowing for capillary blood sampling, a mode of blood withdrawal particularly suitable for the pediatric age group, it could help avoid peripheral venipuncture which is known to cause procedural distress and not rarely requires local anesthesia in infants and children (5).

Therefore, our results strengthen the view of allergen microarrays as promising tools for future low-invasive IgE testing. However, as has been pointed out before (6), further clinical trials with larger numbers of patients are required in order to assess all relevant microarray performance parameters in comparison to established methods of *in vitro* IgE detection.

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References

- Hiller R, Laffer S, Harwanegg C, Huber M, Schmidt WM, Twardosz A et al. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. *FASEB J* 2002;**16**:414–416.
- Kim TE, Park SW, Cho NY, Choi SY, Yong TS, Nahm BH et al. Quantitative measurement of serum allergen-specific IgE on protein chip. *Exp Mol Med* 2002;**34**:152–158.

- Jahn-Schmid B, Harwanegg C, Hiller R, Bohle B, Ebner C, Scheiner C et al. Allergen microarray: comparison of microarray using recombinant allergens with conventional diagnostic methods to detect allergen-specific serum immunoglobulin E. *Clin Exp Allergy* 2003;**33**:1443–1449.
- Deinhofer K, Sevcik H, Balic N, Harwanegg C, Hiller R, Rumpold H et al. Microarrayed allergens for IgE profiling. *Methods* 2004;**32**:249–254.
- Duff AJ. Incorporating psychological approaches into routine paediatric venepuncture. *Arch Dis Child* 2003;**88**:931–937.
- Bacarese-Hamilton T, Gray J, Ardizzoni A, Crisanti A. Allergen microarrays. *Methods Mol Med* 2005;**114**:195–207.

A commercial histamine release test and autologous serum skin test in the diagnosis of autoimmune urticaria

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Key words: autoimmune urticaria; autologous serum skin test; histamine release test.

Autoimmune chronic urticaria, in which functional autoantibodies against the high-affinity IgE receptor Fc epsilon R1 arise, is routinely diagnosed using the autologous serum skin test (ASST) (1, 4). However, the continuous need

of medication by these patients often restricts the use of the ASST. Antihistamines, immunosuppressants and antidepressants may suppress positive results. In addition, ASST is not always assessable because of dermatographism. The gold standard laboratory test for autoimmune chronic urticaria is the demonstration and measurement of histamine release from target basophils

of dermal mast cells (2). A commercial histamine release test (HR-Test; RefLab, Copenhagen, Denmark) has become available.

We studied 10 patients with chronic urticaria and no underlying infections with both the ASST and the HR-Test. The age, sex, type of urticaria, underlying diseases and the medication used by the patients are shown in Table 1. Antihistamines were withdrawn 5 days prior to skin tests except for patient number 8, who could not stop levosetirizine medication because of intensive itching. Serum aliquots from the patients were frozen and sent to RefLab. Fifty per cent, 25% and 12.5% serum dilutions were incubated with dextran-sedimented basophils from healthy donors and histamine release of over 12% was considered positive. The donor basophils were selected on the basis of a histamine release response to anti-IgE and a panel of positive donors releasing over 30% of the cellular histamine content. Histamine was measured fluorometrically by a glassfibre method (3). For the ASST, 0.05 ml of autologous serum was injected intracutaneously into the forearm of each patient (4). The diameter of the wheal was measured after 2 h. The saline controls did not show any wheal reactions.

The sera of four patients showed a positive HR-Test result while only two patients showed a positive ASST (Table 1). All the positive HR-Test reactions were detected at the 12.5% serum dilution. The medication used by the patients explains the main differences between the two tests, as donor basophils used in the HR-Test are not inhibited. To our knowledge, comparisons between the ASST and the commercial HR-Test of Reflab have not been published. However, histamine release has been measured for scientific purposes in earlier studies (4, 5). Over 50% of the patients with positive results in the histamine release test were also positive in the ASST.

The HR-Test is thus warranted in the diagnosis of autoimmune urticaria with continuous medication such as antihistamines or corticosteroids and with dermatographism.