

Original article

Screening for allergic respiratory disease in the general population with the ADVIA Centaur® Allergy Screen Assay

Background: In patients in whom the clinical indication for immunoglobulin E (IgE)-mediated allergic respiratory disease is weak, a single qualitative multiallergen-screening assay for IgE antibody to multiple allergen specificities may support the absence of IgE-mediated allergic respiratory disease. The aim was to investigate the diagnostic efficacy of a new multiallergen-screening assay in relation to skin prick test (SPT) reactivity and objective diagnoses of allergic respiratory disease in a general population setting.

Methods: A total of 709 participants in a population-based study were examined by questionnaire and SPT. Serum was analysed by using a multiallergen-screening assay: the ADVIA Centaur® Allergy Screen (AS) assay. The dichotomized result of the AS assay was compared with SPT reactivity, specific IgE positivity, and a clinical diagnosis of allergic rhinitis or allergic asthma defined by the presence of relevant symptoms and positive SPTs.

Results: Sensitivity, specificity, and positive (PPV) and negative predictive values (NPV) of the AS against SPT reactivity were 86%, 96%, 94%, and 89%, respectively. A negative AS assay test was able to exclude allergic rhinitis and allergic asthma with a probability of more than 96% and 98% (NPV), respectively. The AS assay was able to identify more than 92% and 92% (sensitivity) of cases of allergic rhinitis and allergic asthma, respectively.

Conclusions: The AS assay proved to be a valid measure of allergic respiratory disease and may be used as a screening tool to rule out allergic respiratory disease, and as an objective measure of allergic respiratory disease in epidemiological studies.

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Respiratory diseases represent a significant number of consultations. Efficient diagnostic measures are needed to evaluate these patients. A specific diagnosis of immunoglobulin E (IgE)-mediated allergic respiratory disease (1) may facilitate the initiation of a specific treatment (2). Based on the case history and clinical examination alone it is often difficult to distinguish between IgE-mediated and non-IgE-mediated allergic respiratory disease. When IgE-mediated allergic respiratory disease is suspected, measurement of skin prick test (SPT) reactivity or specific IgE in serum against relevant and available allergens are considered first-line diagnostic procedures (2). Conversely, in patients in whom the clinical indication for IgE-mediated allergic respiratory disease is weak, a single qualitative multiallergen-screening assay for IgE antibody to multiple allergen specificities can support the absence of IgE-mediated allergic respiratory disease (3). Such a test provides a dichotomous (positive or negative) result. The negative predictive value (NPV) of this single test is generally higher than total IgE or any single specific IgE antibody measurement (4).

Validated objective measures of allergic respiratory disease are furthermore needed for epidemiological studies, which may increasingly be based on stored serum samples from biobanks. Measures used for this purpose should prove reasonably sensitive and specific in relation to a clinical diagnosis or gold standard.

The aim of this study was to investigate the diagnostic efficacy of a new multiallergen-screening assay in relation to SPT reactivity and objective clinical diagnoses of allergic respiratory disease in a general population setting.

Methods

Study population

Subjects included in these analyses participated in the first follow up of a prospective cohort study in adults: the Copenhagen Allergy Study (5). The baseline study was established in 1990 and was conducted according to a two-stage protocol (6). In the first stage, a screening questionnaire on respiratory symptoms was mailed to a

random sample of 15–69 year olds living in Copenhagen, Denmark (response rate: 87%). In the second stage, a random group and a symptom group (respondents who had reported respiratory symptoms on exposure to either pollen or furry animals in the screening questionnaire) selected among the respondents were invited to a health examination. Subsequently, 599 (participation rate: 75.5%) and 635 (participation rate: 80.6%) subjects were examined in the random and the symptom group, respectively. A total of 122 subjects belonged to both groups. Thus, a total of 1112 subjects were examined.

At follow up, a total of 1064 of the participants from the baseline study were invited to a re-examination; 734 subjects (69.0% of the invited) were re-examined between October 1997 and November 1998. Characteristics of participants and nonparticipants to the follow-up study have previously been described (5). These 734 subjects were eligible for the present analyses. The ethics committee of Copenhagen County approved the study.

The ADVIA Centaur® Allergy Screen Assay

The ADVIA Centaur® Allergy Screen (AS) Assay (Bayer HealthCare Diagnostics Division, Tarrytown, NY, USA) was used for the qualitative detection of IgE antibodies specific to common inhalant allergens in serum. The test encompasses the 19 most commonly encountered inhalant allergens: two house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), three animal danders (horse, cat and dog), two grasses (*Phleum pratense* and *Cynodon dactylon*), three moulds (*Cladosporium herbarium*, *Aspergillus fumigatus* and *Alternaria alternata*), four trees (*Betula verucosa*, *Quercus alba*, *Olea europae* and *Cryptomeria japonica*), four weeds (*Amrosia artemisifolia*, *Artemisia vulgaris*, *Plantago lanceolata* and *Parietaria officinalis*) and finally one insect (*Blattella germanica*).

In brief, the assay is an IgE capture sandwich immunoassay using direct chemiluminometric technology. The IgE antibody in the patient sample is captured by a monoclonal mouse antihuman IgE antibody covalently coupled to paramagnetic particles in the solid phase. Non-IgE molecules are removed by washing. A composition of biotinylated allergens is added in excess and bind to the allergen-specific IgE antibody captured on the solid phase. Acridinium ester-labelled streptavidin in the Lite Reagent binds to the biotinylated allergen. A direct relationship exists between the amount of allergen-specific IgE in the patient sample and the amount of relative light units detected by the system (7). The system reports AS assay results as index values. Samples with an index value ≥ 1.0 are considered positive for inhalant allergen-specific IgE antibodies. A sample volume of 50 μ l is used per test.

Specific IgE measurements

The serum samples were analysed for specific IgE to birch, grass (timothy), mugwort, dog, cat and mite (*D. pteronyssinus*) with the ADVIA Centaur® immunoassay system (Bayer HealthCare Diagnostics Division) as previously described (7). The analysis for specific IgE was judged to be positive if the measurement was in excess of 0.35 kU/l. Specific IgE positivity was defined as at least one positive test for specific IgE against the allergens tested.

Skin prick tests

The Soluprick SQ® system (ALK-Abelló, Denmark) was used for SPT, as previously described (5). The panel of allergens comprised birch pollen (*B. verucosa*), grass pollen (*P. pratense*), mugwort

pollen (*Ar. vulgaris*), animal danders (horse, cat and dog), two house dust mites (*D. pteronyssinus* and *D. farinae*) and two moulds (*Cl. herbarium* and *Al. alternata*). The participants were asked to abstain from antihistamines for 72 h (astemizole 8 weeks) before the examination. The same technician performed all SPTs. A mean wheal diameter ≥ 3 mm was considered positive. The SPT reactivity was defined as at least one positive SPT.

Clinical diagnoses of allergic rhinitis

Information about respiratory symptoms was obtained in a questionnaire that was completed at home before the examination.

A clinical diagnosis of allergic rhinitis was considered present in subjects with at least one of the following diagnoses.

- 1 Pollen rhinitis: A confirmative answer to the question 'Have you within the last 12 months had an itchy or stuffy nose or have you been sneezing when near grass, trees, or flowers?' and a positive SPT to at least one of birch, grass, or mugwort.
- 2 Animal rhinitis: A confirmative answer to the question 'Have you within the last 12 months had an itchy or stuffy nose or have you been sneezing following exposure to furry animals, e.g. horse, dog, cat, rabbit, guinea pig, or hamster?' and a positive SPT to at least one of horse, dog, or cat.
- 3 Mite rhinitis: A confirmative answer to the question 'Have you within the last 12 months had an itchy or stuffy nose or have you been sneezing following exposure to dust when cleaning rooms or making beds, or when in bed?' and a positive SPT to at least one of two dust mites.

Clinical diagnoses of allergic asthma

A clinical diagnosis of allergic asthma was considered present in subjects with at least one of the following diagnoses.

- 1 Pollen asthma: A confirmative answer to the question 'Within the last 12 months have you experienced shortness of breath or trouble breathing following exposure to grass, trees, or flowers?' and a positive SPT to at least one of birch, grass, or mugwort.
- 2 Animal asthma: A confirmative answer to the question 'Within the last 12 months have you experienced shortness of breath or trouble breathing following exposure to furry animals, e.g. horse, dog, cat, rabbit, guinea-pig, or hamster?' and a positive SPT to at least one of horse, dog, or cat.
- 3 Mite asthma: A confirmative answer to the question 'Within the last 12 months have you experienced shortness of breath or trouble breathing following exposure to dust when cleaning rooms or making beds, or when in bed?' and a positive SPT to at least one of two dust mites.

Analysis

A total of 709 subjects had complete data on all variables and were included in the analyses. The sensitivity, specificity, NPV and positive predictive value (PPV) of the AS assay against each of the clinical diagnoses of allergic rhinitis (or asthma) was calculated (8). The following definitions were employed.

- 1 NPV: The proportion of subjects without allergic rhinitis (or SPT reactivity) among subjects with a negative AS assay test.
- 2 PPV: The proportion of subjects with allergic rhinitis (or SPT reactivity) among subjects with a positive AS assay test.

- 3 Sensitivity: The proportion of subjects with a positive AS assay test among subjects with allergic rhinitis (or SPT reactivity).
- 4 Specificity: The proportion of subjects with a negative AS assay test among subjects without allergic rhinitis (or SPT reactivity).
- 5 Accuracy: The proportion of all test results, both positive and negative that are correct.

Calculation of 95% confidence intervals (CI) was based on the normal approximation (9).

Results

The overall prevalence of a positive AS assay test, a clinical diagnosis of allergic rhinitis, a clinical diagnosis of allergic asthma, and SPT reactivity was 40.6% (288 of 709), 31.2% (221 of 709), 12.0% (85 of 708), and 44.7% (317 of 709), respectively. In the random group, these figures were 23.5% (88 of 374), 13.9% (52 of 374), 4.3% (16 of 374), and 28.1% (105 of 374), respectively.

Table 1 shows the diagnostic efficacy of the AS assay in relation to a clinical diagnosis of allergic rhinitis including subgroups of allergic rhinitis. The NPV was more than 96% for all diagnoses of allergic rhinitis. Thus, a negative AS assay test was able to exclude a clinical diagnosis of allergic rhinitis with a probability of 96.2% (95% CI: 94.4–98.0). The PPV of the AS assay against specific diagnoses of allergic rhinitis was in general considerably lower which is in accordance with the fact that the AS assay test result does not include information about the specific type (pollen, dander, or mite) of sensitization. However, the PPV against a clinical diagnosis of allergic rhinitis, i.e. at least one of the three diagnoses of allergic rhinitis, was 71.2% (95% CI: 66.0–76.4). The sensitivity was high, and the AS assay was thus able to identify

92.8% (95% CI: 89.4–96.2) of subjects with a clinical diagnosis of allergic rhinitis in this general population sample (Table 1). When restricting these analyses to subjects in the random group, the sensitivity, specificity, NPV, and PPV of the AS assay against a clinical diagnosis of allergic rhinitis were 88.5%, 87.0%, 97.9%, and 52.3%, respectively. For allergic asthma the NPV and sensitivity of the AS assay were essentially similar (Table 2).

In addition, the AS assay showed good agreement with SPT reactivity: overall sensitivity 85.8% (95% CI: 82.0–89.6; Table 1). The sensitivity of the AS assay against SPT reactivity increased with increasing number of positive SPTs indicating a dose–response relationship between SPT reactivity and the prevalence of a positive AS assay test (Fig. 1). The AS assay was positive in all subjects with four or more positive SPTs. Moreover, the prevalence of SPT reactivity (Fig. 2) and a clinical diagnosis of allergic rhinitis (data not shown) increased with increasing index value (the test output) of the AS assay indicating that the output of the AS assay may be used as a semiquantitative measure of allergic respiratory disease. In the random group, the sensitivity, specificity, NPV, and PPV of the AS assay against SPT reactivity were 77.1%, 97.4%, 91.6%, and 92.0%, respectively. The agreement between AS assay test results and specific IgE positivity was high, i.e. the accuracy was 94.8% (Table 1). The plot of the AS index value against the sum of concentrations of specific IgE measurements showed a dose–response relationship with a Spearman rank correlation of 0.78 (95% CI: 0.75–0.81; Fig. 3).

The diagnostic efficacy of the AS assay against all of the three symptoms of allergic rhinitis was reasonably

Table 1. Diagnostic efficacy of the ADVIA Centaur® Allergy Screen Assay in relation to clinical diagnoses of allergic rhinitis (for definition of diagnoses see text) and SPT reactivity

Reference (gold standard)	NPV	PPV	Sensitivity	Specificity	Accuracy
Pollen rhinitis	98.1 (413/421)	61.1 (176/288)	95.7 (176/184)	78.7 (413/525)	83.1 (589/709)
Animal rhinitis	99.5 (419/421)	23.3 (68/288)	97.1 (68/70)	65.6 (419/639)	68.7 (487/709)
Mite rhinitis	98.3 (414/421)	14.6 (42/288)	85.7 (42/49)	62.7 (414/660)	64.3 (456/709)
Allergic rhinitis (any of above)	96.2 (405/421)	71.2 (205/288)	92.8 (205/221)	83.0 (405/488)	86.0 (610/709)
SPT reactivity*	89.3 (376/421)	94.4 (272/288)	85.8 (272/317)	95.9 (376/392)	91.4 (648/709)
Specific IgE positivity†	96.2 (405/421)	92.7 (267/288)	94.3 (267/283)	95.1 (405/426)	94.8 (672/709)

NPV, negative predictive value; PPV, positive predictive value; SPT, skin prick test; IgE, immunoglobulin E.

*A positive (≥ 3 mm) SPT to at least one of 10 inhalant allergens.

†A positive test (>0.35 kU/l) for specific IgE against at least one of six allergens.

Table 2. Diagnostic efficacy of the ADVIA Centaur® Allergy Screen Assay in relation to clinical diagnoses of allergic asthma (for definition of diagnoses see text)

Reference (gold standard)	NPV	PPV	Sensitivity	Specificity	Accuracy
Pollen asthma	99.5 (420/422)	17.1 (49/287)	96.1 (49/51)	63.8 (420/658)	66.1 (468/708)
Animal asthma	100 (422/422)	12.9 (37/287)	100 (37/37)	62.8 (422/672)	64.7 (458/708)
Mite asthma	98.8 (417/422)	9.4 (27/287)	84.4 (27/32)	61.6 (417/677)	62.6 (443/708)
Allergic asthma (any of above)	98.6 (416/422)	27.5 (79/287)	92.9 (79/85)	66.7 (416/624)	69.8 (494/708)

NPV, negative predictive value; PPV, positive predictive value.

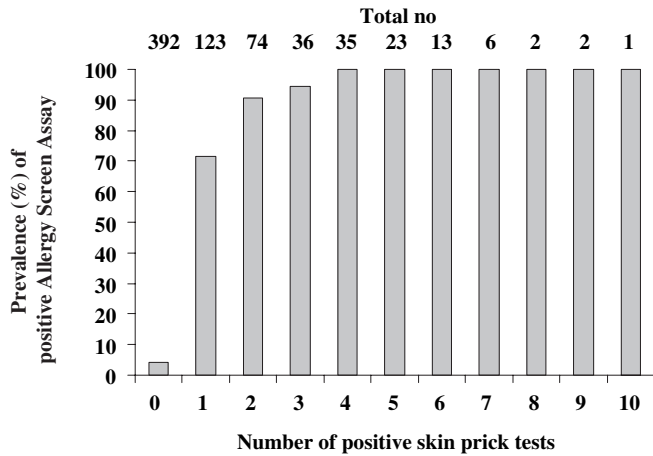


Figure 1. The prevalence (in %) of a positive ADVIA Centaur® Allergy Screen Assay test according to number of positive skin prick tests (mean wheal diameter ≥ 3 mm).

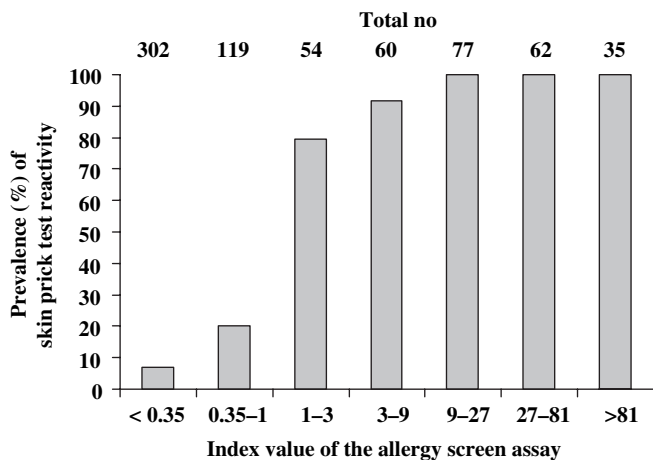


Figure 2. The relationship between the semiquantitative test result (the index value) of the ADVIA Centaur® Allergy Screen Assay and the prevalence of skin prick test reactivity (a positive skin prick test against at least one of 10 inhalant allergens).

similar to the diagnostic efficacy of SPT reactivity against these symptoms (data not shown).

Discussion

We found that the AS assay was a valid measure of allergic respiratory disease. Thus, a negative test result was able to exclude a clinical diagnosis of allergic rhinitis with a probability of 96%, and the AS assay was able to identify 92% of all cases of allergic rhinitis in this population. These figures were similar for allergic asthma.

The PPV and NPV of any diagnostic test are dependent on disease prevalence in the population under study (8).

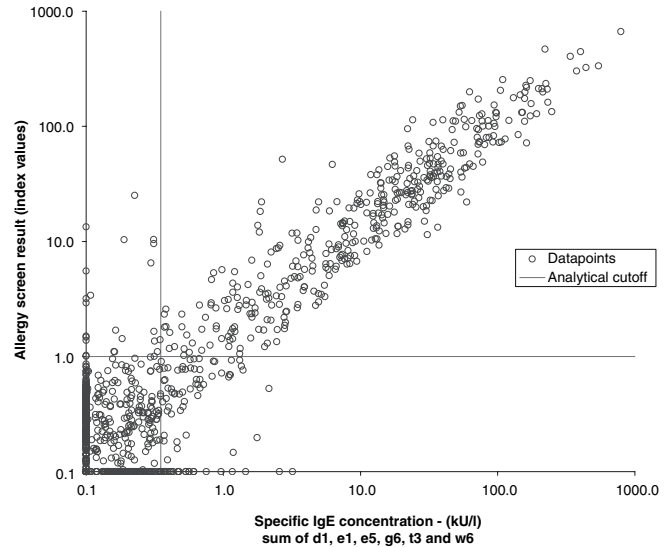


Figure 3. The correlation between the sum of concentrations of measurements specific immunoglobulin E (IgE) against six inhalant allergens and the index value of the Allergy Screen Assay.

In line with this, we found that the PPV was considerably lower in the random group, where disease prevalence was lower, when compared with the total population (52.3% in the random group vs 71.2% in the total population). However, the NPV was slightly higher in the random group (97.9% in the random group vs 96.2% in the total population). As expected the sensitivity (88.5% vs 92.8%) and specificity (87.0% vs 83.0%) were reasonably similar in the random group and the total population.

The pattern of sensitization may vary with geographic and climatic factors. Therefore, the panel of allergens included in the multiallergen assay in the present study might not cover all relevant allergens in all settings, and additional allergens might be required.

In general, multiallergen screening assays are interpreted qualitatively as a dichotomous test response. Our data suggests that the output of the AS assay may be interpreted semiquantitatively, as the probability of SPT reactivity increases with increasing test output (Fig. 2). This feature may be further developed and used for research purposes or even in clinical practice.

Skin testing requires training and experience to perform with both accuracy and consistency, and for interpretation of results (10). Furthermore, costs of keeping allergen extracts in stock may be a disadvantage when test volume is low. The use of a multiallergen *in vitro* test does not require a trained technician or a clinician with allergological experience and training and may therefore be used in general practice when these resources are not available. The AS assay should not be used as the only diagnostic test in cases with probable IgE-mediated allergic respiratory disease. In these cases knowledge of specific sensitizations is often required and

measurement of SPT reactivity or specific IgE should still be used as primary paraclinical procedure.

An increasing number of biobanks are becoming available for research purposes. In the present population-based study, the AS assay proved to be a valid measure of both SPT reactivity and allergic respiratory disease defined by objective criteria. Thus, the AS may be used as an objective measure of allergic respiratory disease in epidemiological studies based on stored serum samples. The use of a multiallergen assay in these studies may have several advantages: (i) it requires a small quantity of serum (50 µl), which is important, when there is a limited quantity of serum available. (ii) Compared with SPT, blood sampling does not require a trained technician. (iii) Furthermore, in multicentre studies serum samples may be analysed at a central laboratory, thereby avoiding interlaboratory or interobserver variability. In such studies significant variations in SPT results between fieldworkers at different centres have been reported (11). Ongoing large-scale population-based studies at the Research Centre for Prevention and Health, Copenhagen, Denmark, have included measurement of IgE-mediated allergy in serum samples from the biobank of this institution by using the AS assay. Quality control data

from this biobank has previously confirmed the stability of specific IgE antibodies in serum samples stored for 8 years (12).

In conclusion, the AS assay is a valid screening tool for allergic respiratory disease. The AS assay yields high NPV even in a population with a high prevalence of allergic respiratory disease and it therefore has the potential to be the primary test to rule out IgE-mediated allergic respiratory disease. Furthermore, the AS assay may be used as an objective measure of IgE-mediated allergic respiratory disease in population-based epidemiological studies.

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