

## Original article

# Long-term repeatability of the skin prick test is high when supported by history or allergen-sensitivity tests: a prospective clinical study

**Background:** Long-term reproducibility of the skin-prick test (SPT) has been questioned. The aim of the study was to investigate the clinical relevance of SPT changes.

**Methods:** SPT to 10 common inhalation allergens was performed annually from 1999 to 2001 in 25 nonsensitized and 21 sensitized subjects. An SPT was positive when  $\geq 3$  mm, and repeatable if either persistently positive or negative. Clinical sensitivity to birch pollen was used as model for inhalation allergy, and was investigated at inclusion and at study termination by challenge tests, intradermal test, titrated SPT and IgE measurements. Birch pollen symptoms were confirmed in diaries.

**Results:** The repeatability of a positive SPT was 67%, increasing significantly to 100% when supported by the history. When not supported by history, the presence of specific IgE was significantly associated with a repeatable SPT. Allergen sensitivity was significantly lower in subjects losing SPT positivity. The repeatability of a negative test was 95%, decreasing significantly to 87% by the presence of other sensitization. Development of a positive SPT was clinically relevant. Elevation of SPT cut-off point did not enhance repeatability.

**Conclusion:** SPT changes are clinically relevant. Further studies using other allergens are needed. Long-term repeatability of SPT is high in the presence of a supportive history.

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Skin prick test (SPT) is considered a valid, safe, low-cost and easy-to-perform method to demonstrate IgE-sensitization towards aeroallergens (1, 2). It is recommended as the allergological 'test of choice' in clinical practice as well as in clinical and epidemiological research (3, 4). Short-term reproducibility is high and adequately examined whereas long-term reproducibility using unchanged batch of allergen extracts is lower and less established (5–11). Reproducibility data are rarely available, but data on repeatability are present in – or can be calculated from – a few studies (6–12). Repeatability, or persistency, is a measure of the reproducibility of the initial qualitative outcome, i.e. the positive or negative SPT (7). The median repeatability of a positive SPT is 87.0% (range 62.1–95.8%), and 86.8% (range 77.4–93.6%) for a negative SPT (6–11). This suggests that both losses and onsets of skin sensitization are expectable in long-term follow-up studies. In epidemiological studies, the incidence of new skin sensitization is an established measure for the increase of

atopy in a population (8–10, 13). Onset of new sensitization has been found to be more frequent in subjects with pre-existing sensitization than in nonsensitized subjects (7, 14). Loss of SPT positivity has attracted little attention, mostly considered a technical problem related to extract, performer, and/or patient (6). Increasing the cut-off limit from the recommended  $\geq 3$  to  $\geq 4$  mm was reported to exclude loss of positive SPT reactions (9). Loss of SPT positivity has been associated with lower total IgE levels than in persistently SPT-positive subjects (10). However, no studies have investigated the clinical relevance of SPT changes by challenge tests or completion of symptom diaries. The aim of the present study was to evaluate long-term repeatability of the SPT in a community-based population during 3 years, and to relate SPT repeatability to clinical allergen sensitivity.

## Methods

### Design

In a cohort of adults recruited from a community-based population, skin sensitization was annually investigated four times (Fig. 1) with

*Abbreviations:* CPT, conjunctival challenge test; IgE, immunoglobulin E; LPR, late-phase reaction; NPT, nasal challenge test; SPT, skin prick test.

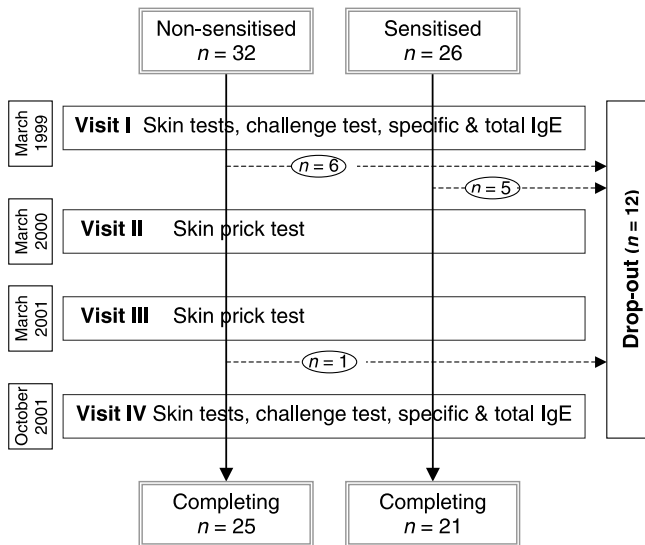


Figure 1. Study design, and flow of patients.

a panel of 10 inhalation allergens (Fig. 2), common in tempered areas of the Northern Hemisphere (11, 15, 16). The clinical relevance of skin sensitization was investigated at inclusion (visit I) and at study termination (visit IV) with a variety of allergological tests (Fig. 1).

#### Model allergen

Sensitivity to birch pollen was used as model based on the need for reporting of relevant symptoms, and on the use of a stable allergen extract. During the birch pollen season in Denmark (late March to early May), no relevant concomitant allergen season is present, thus allergic symptoms during this period can exclusively be related to birch, which elevates the specificity of a positive history (17, 18). Stable allergen extracts are commercially available, and are widely used in diagnostic and therapeutic, as well as in practical and scientific allergology (11, 18).

#### Study population

Fifty-eight participants were recruited in February 1999 from a population of 1000 interviewed and skin-prick tested adults. Inclusion criteria were either a positive SPT to birch and/or nine other common inhalation allergens (sensitized group), or a negative skin prick test supported by no history of previous or current episode of any atopic disorder (nonsensitized group). Exclusion criteria were perennial rhinitis or asthma, pregnancy or lactation, and malignancy or other severe systemic disease. A positive history of birch pollen allergy was defined as more than 4 days of symptoms reported in daily diary cards (on both symptoms and medication use) during the local birch-pollen season 1999 (March–May). The season was defined as the period covering the mid-90% of the total birch pollen count (19). The symptom/medication rating scale and data on symptoms during the study period are presented elsewhere (16). To avoid masking of possible symptoms, corticosteroids were prohibited (19). The study was approved by the local ethics committee of Copenhagen, Denmark, and written informed consent was obtained from all participants prior to admission to the study.

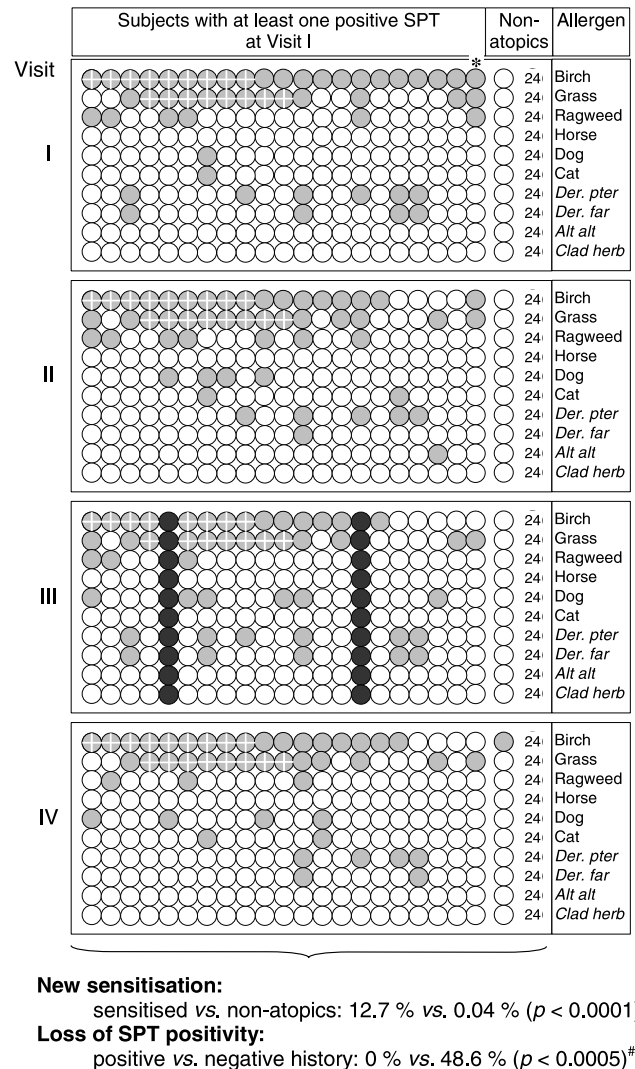


Figure 2. The results and repeatability of allergen skin-prick tests (SPT) during the four study visits using the SPT cut-off value of  $\geq 3$  mm. The prevalences of onset and loss of skin sensitization are shown below the figure (observe that prevalences of onset and loss of a positive allergy history are published elsewhere (16). Each column depicts the SPT results of a single patient, and rows the allergens used. A white circle indicates a negative SPT, a grey circle a positive SPT, and a black circle a missing test. A white cross indicates symptoms at visit I, and '24' indicate that 24 participants had identical courses that are shown in a single column. \*Patient suffering from atopic dermatitis. <sup>#</sup>Fisher's exact test.

#### Allergen extracts

Commercially available aqueous allergen solutions produced according to the international guidelines on allergen preparation were used: Soluprick® (10 HEP/ml) for SPT, and dilutions of birch Aquagen® for other skin tests and challenge tests (both: 12.5 µg Bet v 1/ml; ALK-Abellø, Hørsholm, Denmark) (5). All extracts were kept at 4°C, and handled as recommended by the manufacturer. Vials were discharged when expired, leading to a batch shift of

Soluprick between visit II and III, whereas all other tests were performed with the same allergen batch throughout the study. Use of antihistamines was discouraged 1 week prior to each visit. All tests were performed by one study nurse and UB (exception: CRJ replaced UB at screening and visit I).

Skin prick test with 10 allergens

The SPT was performed in duplicate on the volar side of the antebrachium with the allergens listed in Fig. 2. According to international guidelines, positivity was defined as a mean wheal diameter  $\geq 3$  mm (3, 4). Histamine dihydrochloride 10 mg/ml was used as positive control, and diluent solution as negative control. All SPT performers in the study fulfilled the internal reproducibility standard of SPT with histamine dihydrochloride: a standard deviation  $< 40\%$  (single concentration: 10 mg/ml), and a correlation coefficient  $> 0.85$  (titrated SPT).

Skin prick tests and intradermal test to birch pollen

Loss of an initially positive birch SPT resulted in an immediate repetition of the SPT on the opposite arm. At visit IV, a titrated SPT (t-SPT) was performed using concentrations of 1.25, 3.75 and 12.5  $\mu\text{g}$  Bet v 1/ml (titres 1–3), proceeding to 37.5 and 125  $\mu\text{g}$  Bet v 1/ml (titres 4 and 5) in subjects without self-reported birch pollen allergy. Titres 4 and 5 were included to avoid a negative reaction due to insufficient allergen presentation to mast-cell bound IgE (4). Results were expressed as the lowest titre producing a positive reaction (a mean wheal diameter  $\geq 3$  mm), or as  $5^+$  if no positive reactions. In all sensitized subjects, the intradermal late-phase wheal-and-flare reaction (LPR) at visits I and IV was recorded 18–24 h after monoplicate, intradermal injection of 0.05 ml of 125 ng Bet v 1/ml, and a negative control on the opposite arm (19). The LPR reaction was considered positive if the allergen mean wheal diameter minus the negative control exceeded 5 mm (5).

Birch pollen challenge tests

At visits I and IV, titrated conjunctival (CPT) and nasal (NPT) challenges were performed in logarithmic steps from 12.5 ng to 12.5  $\mu\text{g}$  Bet v 1/ml (titres 1–4), proceeding to 125  $\mu\text{g}$  Bet v 1/ml (titre 5) in subjects without self-reported birch pollen allergy, or until a positive test (19). A test was considered negative if the highest allergen concentration did not elicit significant clinical symptom. The result was expressed as the highest titre tolerated (20).

Paraclinical investigations

Blood samples for IgE measurements were drawn at visit I and IV. Specific IgE (s-IgE) levels  $\geq$  CAP class 2 was considered positive (CAP System; Pharmacia Diagnostics, Uppsala, Sweden). Total IgE was measured by a micro-particle enzyme immunoassay (IMX System; Abbott Diagnostics, Abbott Park, IL, USA).

Statistical analysis

Statistical analyses were performed with computer software (SPSS, release 10.0.7; SPSS, Chicago, IL, USA), using the Mann–Whitney *U*-test (continuous variables), and Fisher’s exact test, and odds ratio (OR, categorical variables) (4, 21). Data were described using frequency distributions, median, range, and ratios. Statistical significance was defined as  $P < 0.05$  (21). Participants not attending

visit IV were excluded. Missing data were omitted from the final calculations, thus no fitted values were constructed (21). If more than 10% of data were missing in the diaries, data analysis was considered inconclusive.

Results

Subjects

Forty-six participants completed the study (Table 1). One subject in the sensitized group suffered from mild atopic dermatitis on elbows and hands. He had a total IgE level of 1095 kU/L, and his SPT is shown in Fig. 2. Twelve subjects were excluded, as they did not attend visit IV (Fig. 1). The excluded subjects were evenly distributed between the groups, and no significant demographic differences were present between excluded and attending subjects (data not shown). Diaries contained less than 7% missing data, and no diary contained information on medicine intake without concomitant symptom registration.

Long-term repeatability of SPT

Figure 2 depicts the incidences of onset or loss of sensitization during the study. The overall incidences of onset and loss were 5.1 and 32.7%, respectively. All histories of allergy symptoms were accompanied by a positive SPT thus no onset of sensitization was preceded by a positive history. The 38 nonrepeatable tests were experienced by 20 subjects with a median of two (range 1–4) SPT reactions. The number of SPT changes between visits I & II, II & III, and III & IV were insignificantly different (*n* losses: 9, 10 and 15; *n* onsets of sensitization: 11, 11 and 10, respectively).

When the history and SPT disagreed (a negative history, a positive SPT), a persistently positive SPT reaction was significantly associated with positive levels of specific IgE ( $P < 0.01$ ; data not shown). On the contrary, when the history and SPT agreed, additional measurement of did not significantly add prognostic

Table 1. Demographic and clinical baseline data (visit I)

|                                        | Nonsensitized<br>( <i>n</i> = 25) | Sensitized<br>( <i>n</i> = 21) | <i>P</i> -value‡ |
|----------------------------------------|-----------------------------------|--------------------------------|------------------|
| Age, years (range)                     | 22 (20–26)                        | 24 (20–41)                     | 0.03             |
| Female/male, <i>n</i>                  | 9/16                              | 7/14                           | $>0.9$           |
| SPT $\geq 3$ mm, <i>n</i>              | 0                                 | 52                             | –                |
| Allergies, <i>n</i>                    | 0                                 | 17                             | –                |
| Allergic subjects, <i>n</i>            | 0                                 | 11                             | $<0.0001$        |
| Birch pollen allergy, <i>n</i> *       | 0                                 | 9                              | $<0.001$         |
| SPT $\geq 3$ mm, <i>n</i> per subject† | 0                                 | 2 (1–4)                        | –                |

\* As reported in diary.

† median (range).

‡ Categorical data: Fisher’s exact test; Continuous data: Mann–Whitney *U*-test.

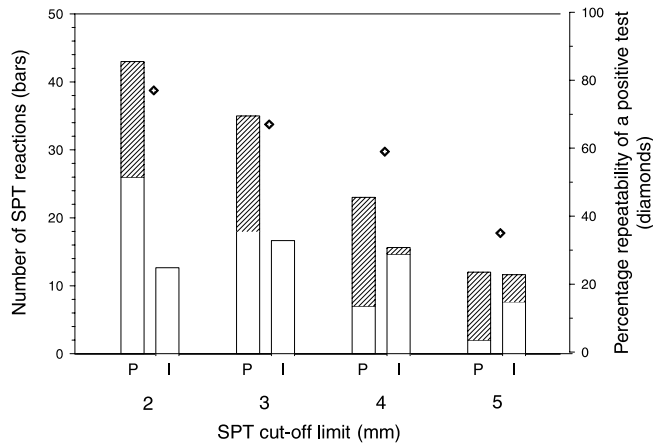


Figure 3. The impact of skin-prick test (SPT) cut-off limits on the repeatability of a positive SPT. The higher the cut-off limit, the fewer positive SPT reactions at visit I. Bars: skin prick tests accompanied (shaded fields) and not accompanied (white fields) by a positive history of allergy. Diamonds: repeatability of a positive SPT (right Y-axis). Abbreviations: P, persistently SPT positive (i.e. four consecutively positive SPT); I, intermittently SPT positive (i.e. initially positive SPT becoming negative at least once during the three subsequent tests).

information to SPT repeatability (negative and positive match between S-IgE and history:  $P > 0.6$ ).

The SPT reactions  $\geq 5$  mm were more likely to be persistently positive (Fisher's exact test:  $P < 0.0005$ ). The diameter of the initial test was significantly larger when the subsequent tests were persistently positive than when SPT positivity was lost [5.5 mm (range 4.0–13.5) vs 3.5 mm (range 3.0–6.5);  $P < 0.00005$ , Mann-Whitney  $U$ -test; data not shown].

Concerning an initially negative SPT, repeatability was significantly lower in subjects with other sensitization (Fig. 2; Fisher's exact test:  $P < 0.00001$ ). Correspond-

ingly, pre-existing sensitization was a risk factor for new sensitization (OR 35.7, 95% confidence interval 4.8–250).

#### Changing SPT cut-off limit

Figure 3 depicts the repeatability of the initial positive SPT reaction with changing SPT cut-off points (from 2 to 5 mm). Both the number of positive reactions and the repeatability decreased. The number of persistently positive SPT reactions was significantly higher using 2- or 3-mm cut-off points than 5 mm ( $P < 0.05$ ; Fisher's test). The repeatability of a negative test was 94, 95, 96 and 97% at 2, 3, 4 and 5 mm cut-off limits (insignificant differences). Choosing a cut-off limit  $\geq 4$  mm implicated loss of clinically relevant SPT reactions (positive history and clinical reactivity at allergen challenge; Fig. 3).

#### Clinical characterization of subjects losing a positive birch pollen SPT reaction

Twenty-one subjects were SPT positive to birch at visit I, and five of these subjects had a negative birch SPT at least once during visits II to IV. None of these negative reactions were positive when immediately repeated. Table 2 shows that the subjects experiencing a loss of birch SPT positivity ('reverters') as early as visit I were significantly less birch-pollen sensitive than persistently SPT-positive subjects. Neither age nor gender differed significantly (data not shown). Two of these 'reverters' were birch pollen sensitive: both were positive to the highest titres of CPT and t-SPT, and one reported allergy symptoms once during the study period. Experimentally, the 'reverters' resembled the nonsensitized group except by having a significantly higher number of positive SPT reactions, and a higher total IgE (data not shown).

All birch-SPT negative subjects were negative in both skin tests and in challenge tests.

Table 2. Differences in clinical and paraclinical birch pollen sensitivity at visits I and IV between subjects with intermittently and persistently positive birch SPT

|                         | Visit I               |                      |                       | Visit IV                           |                     |                       |
|-------------------------|-----------------------|----------------------|-----------------------|------------------------------------|---------------------|-----------------------|
|                         | Intermittent§ (n = 5) | Persistent¶ (n = 16) | Difference (P-value)‡ | Intermittent (n = 5)               | Persistent (n = 16) | Difference (P-value)‡ |
| Birch SPT, mm (range)   | 4.5 (3.5–4.5)         | 6.0 (4.0–10.0)       | <0.005                | 0.0 (0–5.5)                        | 5.0 (3.0–9.5)       | <0.001                |
| Hist* SPT, mm (range)   | 8.5 (7.5–9.0)†        | 7.5 (6.0–9.5)†       | >0.2                  | 6.0 (4.5–8.0)†                     | 5.5 (4.0–8.5)†      | >0.3                  |
| t-SPT, titre (range)    |                       | (Not performed)      |                       | 5 <sup>+</sup> (3–5 <sup>+</sup> ) | 1 (1–3)             | <0.05                 |
| LPR, mm (range)         | 0 (0–0)               | 0 (0–96)             | >0.2                  | 0 (0–42)                           | 47 (0–70.5)         | <0.05                 |
| CPT, titre (range)      | 5 (3–5)               | 2 (1–4)              | <0.002                | 5 (4–5)                            | 3 (1–4)             | <0.0001               |
| NPT, titre (range)      | 5 (4–5)               | 3 (1–5)              | <0.005                | 5 (5–5)                            | 3 (2–5)             | <0.002                |
| CAP class (range)       | 0 (0–1)               | 2 (0–4)              | <0.002                | 0 (0)                              | 3 (1–4)             | <0.0005               |
| Total IgE, kU/l (range) | 48 (20–895)           | 96 (21–456)          | >0.6                  | 37 (10–1095)                       | 65 (13–330)         | >0.9                  |

\* Histamine.

† Significant within-group difference between visits I and IV ( $P < 0.05$ ).

‡ Categorical data analysed with Fisher's exact test, and continuous data analysed with paired and unpaired Mann-Whitney  $U$ -test.

§ Initially positive SPT becoming negative at least once during the subsequent tests.

¶ Four consecutively positive SPT.

Clinical characterization of the onset of a positive birch pollen SPT

A single onset of birch positivity occurred during the study (at visit IV), and was accompanied by a positive CPT (highest titre), negative NPT, positive t-SPT (lowest titre), absent history of birch pollen allergy in the study period, and immeasurable levels of specific IgE. From visit I–III, clinical and paraclinical parameters of this subject was within the interquartile range of the parameters of the persistently SPT-negative subjects (data not shown), thus the onset of sensitization was not predicted.

## Discussion

We have in the present study demonstrated that long-term repeatability of a positive SPT reaction was high in the presence of a supportive history, or when the SPT was more than 5 mm in diameter (Figs 2 and 3). Contrary to the other authors, we do not advocate for a general increase of the SPT cut-off limit as both the repeatability and sensitivity decreased with increasing cut-off limit (Fig. 3) (4, 9). Long-term persistency of a negative SPT was high in the present study and was as previously shown associated with absence of other sensitization (Fig. 2) (7, 14). Measuring specific IgE added only prognostic information in case of a mismatch between history and SPT result. This finding could be due to selection criteria or a high probability of detecting s-IgE in allergic subjects (22). In our material, no history of allergy was present without concomitant skin sensitization thus no conclusions could be made on the possible impact of a positive history on long-term repeatability of a negative SPT. Using sensitization to birch pollen as a model for aeroallergen sensitization in general, we found that loss of SPT positivity was accompanied by none to

low allergen sensitivity: i.e. significantly smaller allergen wheals, and tolerance of higher allergen titres at challenge (Table 2). We clearly showed that (low) allergen sensitivity was not a cause of unspecific reactivity, as no nonsensitized subjects had any positive challenge tests. Clinical sensitivity despite an intermittently positive SPT suggests clinically relevant sensitization. This state of fluctuating SPT sensitization has to our knowledge yet only been described, but not clinically investigated, by Kuehr et al. (7). It might represent the initial sensitization to allergen, preceding that of persistent skin-sensitization and serological sensitization (Table 3) (11, 22). Previously, total IgE levels was found to be lower in these subjects, a finding we could not reproduce perhaps because of population differences and/or a type II error (10, 21).

Annual incidences of loss and onset of SPT positivity are present in, or can be calculated from, six population-based studies (Table 3). The study by Horak Jr et al. (12) is not commented on as it was read just before proofreading of the manuscript. The observed differences possibly reflect the heterogeneity of the studies concerning populations, inclusion criteria, allergen extracts, definitions of SPT positivity, follow-up duration, and number of re-tests. Our population-based cohort was selected to ensure comparable groups of nonsensitized and birch-pollen sensitized subjects in a model for clinical sensitivity to an aeroallergen (16). Lack of SPT repeatability can be related to technical impairment or to true changes in the patients' allergic state. Indeed, onset of new sensitization is a generally accepted measure of atopy development in population studies (8–10, 13), and loss of sensitization is considered a technical error (6). The SPT is influenced by extract (inter-batch differences of allergen content, contamination by irritants), performer (insufficient skills, nonstandardized technique), patient (age,

Table 3. Annual incidences of loss and onset of sensitization in published studies with consecutive skin-prick testing

| Author (reference)    | Loss (incidence) | Onset (incidence) | Duration (years) | Number (population)* | Visits (n) | Allergens                 | Cut-off limit†  |
|-----------------------|------------------|-------------------|------------------|----------------------|------------|---------------------------|-----------------|
| Johnston et al. (6)   | 3.4              | 11.3              | 2                | 114 (c)              | 2          | 3 allergens <sup>1</sup>  | ≥3 mm           |
| Kuehr et al. (7)      | 20.7             | 4.8               | 2                | 587 (c)              | 3          | Birch                     | ≥3 mm           |
| Kuehr et al. (7)      | 4.5              | 3.5               | 2                | 587 (c)              | 3          | Grass                     | ≥3 mm           |
| Barbee et al. (8)     | 18.6             | 9.5               | 1                | 2443 (a + c)         | 2          | Grass                     | >1 mm           |
| Peat et al. (9)       | 1.0              | 5.1               | 4                | 380 (c)              | 3          | 13 allergens <sup>2</sup> | ≥4 mm           |
| Oryszczyn et al. (10) | 5.1              | 2.8               | 5                | 223 (male a)         | 2          | 2 allergens <sup>3</sup>  | >saline control |
| Linneberg et al. (11) | 1.6              | 1.7               | 8                | 734 (a)              | 2          | 10 allergens <sup>4</sup> | ≥0.5 × h.w.§    |
| Horak et al. (12)     | 8.3              | 10.9              | 3                | 603 (c)              | 2          | 7 allergens <sup>2</sup>  | ≥2 mm           |
| Bodtger et al.‡       | 9.3              | 1.5               | 3                | 46 (a)               | 4          | 10 allergens <sup>4</sup> | ≥3 mm           |

\* c/a = children/adults.

† Cut-off limit for SPT positivity.

‡ Present study.

§ h.w. = histamine wheal.

<sup>1</sup> Grass, house dust mites, and cat dander.

<sup>2</sup> House dust, house dust mites, animal dander, pollens, and moulds.

<sup>3</sup> Grass and house dust mites.

<sup>4</sup> Soluprick standard panel (allergens listed in Table 2).

antihistamine intake, skin or systemic disease, circadian endogenous secretion of corticosteroids), or external factors (ongoing or recent allergen exposure) (2, 4, 14, 23–26). We have tried to diminish the importance of these factors. We used only standardized, commercially available extracts in the hands of a specialized staff with (short-term) reproducible SPT to histamine dihydrochloride (5). The unavoidable change of primary investigator between visits I and II, and of allergen batch between visits II and III seemed to have had no major implications on the study, as the number of losses and debuts of sensitization varied insignificantly throughout the study. All tests were performed at the same time of the year (except visit IV), were preceded by antihistamine avoidance, and were too close in time to be affected significantly by the age-related decrease in histamine and allergen wheal diameters (25). The participants suffered from no other disease than allergy, and the circadian variations were considered to be of inferior importance (23). Similar to Johnston et al., we observed a decrease in histamine wheal diameter independently of clinical phenotype (Table 2) (6). Theoretically, minor batch-to-batch differences in histamine content, or a time-dependent slow loss of histamine potency in an opened vial could be the cause, but as the histamine results were qualitatively persistent throughout the study, we do not consider this to negatively influence the major conclusions of the study.

The annual loss of SPT positivity is between 1 and 20% in prospectively examined populations (Table 3). The tendency towards higher rates in children could be due to a relative more inert allergen-response in adults, or a 'saturation effect' by which the persons with the highest number of genetic and environmental risk factors develop sensitization earlier in life (27). The same mechanisms might be responsible for the higher incidence of new sensitization in subjects being previously sensitized (Fig. 2) (7, 14).

Future studies might link onset or loss of SPT positivity to individual exposure to relevant allergens to identify exposure thresholds for development of sensitization and symptoms (28, 29). As the exposure to both indoor and outdoor allergens shows huge inter-individual variation within the same community, community-based allergen trapping provides insufficient data, and new modalities for measuring allergen exposure need development (30).

Our results were based on a small population sample that allows both type I and type II errors to be present, thus our data need confirmation in large-scale studies (21). Yet, our study is the first published attempt to relate long-term persistency of the most widely used allergological test to relevant clinical variables in a prospective design. In conclusion, we confirmed the high repeatability of the SPT found in short-time studies (1, 2) but mainly when the history was supportive of the SPT result. Loss of SPT positivity occurred only in asymptomatic subjects, and was associated with absent to low clinical sensitivity to allergen. Onset of new sensitization occurred almost exclusively in subjects sensitized to other aeroallergens, and was associated with low clinical sensitivity to allergen. Future studies are needed to investigate the immunological background for fluctuating sensitization that could represent a very early form of clinical sensitization.

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