

Concordance of Lateral Flow and Skin Prick Tests in the Assessment of Allergen Sensitisation in the Epidemiological Field in Children

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Key Words

Atopy • Children • Diagnosis • Epidemiology • ImmunoCAPTM Rapid • Lateral flow test • Sensitisation • Skin prick test

Abstract

Background: A new lateral flow test (LFT) for office use provides information about sensitisation to common allergens. Concordance between LFT and skin-prick test (SPT) has not been assessed in epidemiological studies. The aim of this study is to determine LFT-SPT concordance in this field.

Methods: Plasma samples (n = 270) from children aged 9–12 years previously SPT tested were used for the analysis. We selected 180 samples from children SPT positive to any of the 6 allergens which were common to SPT and LFT (cat, birch, timothy-grass, olive, pellitory and *Dermatophagoides pteronyssinus*), and 90 samples from children SPT negative. The intensity of the LFT colour line which indicates a positive reaction was rated from 0 to 4. **Results:** Only results on cat, olive and *D. pteronyssinus* were analysed, as only these had an acceptable number of individuals. When atopy was defined as at least 1 wheal with a mean diameter ≥ 3 mm, agreement was excellent (Cohen's $\kappa = 0.81$) when a lightly visible line was considered positive in the LFT, and dropped

substantially (Cohen's $\kappa = 0.68$) when this value of LFT was considered negative. The correlation between the SPT wheal diameter and the intensity of the LFT line was 0.71 for cat, 0.81 for olive and 0.78 for *D. pteronyssinus*. **Conclusions:** As compared to SPT, LFT is a reliable method to screen for sensitisation to cat dander, olive pollen and *D. pteronyssinus* in the epidemiological field among schoolchildren. There is a good correlation between the SPT wheal diameter and the intensity of the LFT line.

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Introduction

The diagnosis of allergic diseases includes the work-up for allergen sensitisation, which should be put in the context of a clinical history. The most common method for this work-up is the skin prick test (SPT), which is a cheap, quick and reliable way to detect allergen-specific IgE to a large number of allergens. Furthermore, SPT is the test most frequently used to define allergen sensitisation in epidemiological studies, both in children and in adults. For instance, a major epidemiological study in children [phase II of the International Study of Asthma and Allergies in Childhood (ISAAC)] used SPT to define sensitisa-

tion in a very large international population of school-children [1]. However, in some circumstances, such as in the primary care or school setting, obtaining a capillary blood sample from a finger might be more convenient than performing a complete SPT test.

A new lateral flow test (LFT) for the assessment of IgE-mediated sensitisation in the office has been recently launched. As no infrastructure is needed, it could be carried out in the primary care or the school setting. It is an in vitro assay that requires a capillary blood drop from the patient. Unlike other diagnostic methods, besides determining solely if the patient is sensitised to a panel of allergens, this LFT also shows information about any specific sensitisation to one or several allergens in a panel of 10 of the most common: cat, birch, mugwort, timothy grass, egg, dog, olive, spreading pellitory, *Dermatophagoides pteronyssinus* and milk. The procedure takes 20 min and it can be performed simultaneously in several individuals.

To the best of our knowledge, there is no study comparing the results of LFT and SPT. There is, however, a study where LFT results are compared with the amount of allergen-specific IgE measured by the CAP system in an immunology laboratory. This study was carried out in children (1–14 years old) who had previously suffered from several episodes of wheezing [2]. Thus, it was not performed in the general population, nor did the authors compared LFT to SPT.

The aim of the present study is to compare the results of LFT and SPT in the paediatric epidemiological field.

Subjects and Methods

Study Population

As part of phase II of the ISAAC study in Spain, the centre in Cartagena recruited 1,471 children aged between 9 and 12 years. Details of sampling and the participation rate have been explained elsewhere [3]. From this sample of 1,471 children, 1,012 authorisations were obtained to carry out SPT (all performed), and 720 to extract blood samples (707 collected through venous puncture). These samples were centrifuged and their plasma stored frozen at -70°C . From the group of children who had taken both tests and from whom plasma samples were available ($n = 621$), 270 plasma samples were selected: 180 from children with at least 1 positive result to any of the 6 allergens which were common to SPT and LFT (*D. pteronyssinus*, birch, timothy grass, olive, spreading pellitory and cat); and 90 with negative results to all the allergens in the SPT. The 180 positive children were those with the most numerous positive wheals in the SPT. The number of children was planned so as to have a 2:1 rate with the controls and to have enough children for allowing establishment of statistically powerful concordances within the most prevalent allergens.

Table 1. LFT scoring system

Value	LFT result	Description
0	negative	no visible line
1	weakly positive	lightly visible line
2	positive	line less intense than control
3	positive	line as intense as control
4	positive	line more intense than control

Skin Prick Test

SPT was performed according to the ISAAC phase II protocol [1] which includes the following allergens (ALK-Abello, Denmark): *D. pteronyssinus*, *D. farinae*, cat, alternaria, mixed trees (birch, alder and hazel) and mixed grasses (orchard, ryegrass, fescue, Kentucky bluegrass, timothy grass and oats). Olive and spreading pellitory (both ALK-Abello, Spain) were added as prevalent allergens of the area. The number of children sensitised only to dog is very low in our environment, so this allergen was not included. All SPTs were performed by a doctor and a nurse. They were trained prior to starting the study by performing 3 series of 16 pricks with histamine (10 mg/ml) on the volar surface of the arm of a volunteer. The coefficient of variation was less than 20% in the last series. The contours of each wheal were outlined and transferred to an adhesive transparent tape for later reading. SPT-positive subjects were defined as those who had at least 1 positive reaction: wheal mean diameter measuring 3 mm or more after subtraction of the negative value [4]. The mean diameter was calculated using the largest and the smallest wheal diameters.

Lateral Flow Test

The LFT testing was carried out by means of the ImmunoCAPTM Rapid device (Phadia Diagnostics, Uppsala, Sweden) following the directions of the manufacturer. The type of LFT used was the ImmunoCAP Rapid 'Wheeze/Rhinitis Child', which includes the following standardised allergens: cat (epithelium and dander), birch, mugwort, timothy grass, egg white, dog (dander), olive, spreading pellitory, *D. pteronyssinus* and cow's milk.

According to the recommendations of the manufacturer, the study was performed using blood plasma. The results of LFT were established visually by 2 different persons, who agreed on the result. Depending on the colour intensity of the LFT line as compared with its respective control line, 5 different types of results were recorded (score 0–4): no line; lightly visible line; and intensities less than, equal to or higher than control (table 1).

The present study also tested if there was any difference between using serum or plasma in a short series of 30 samples. Positive or negative results of each one of the 10 allergens included in the LFT panel were compared.

Statistical Analysis

Agreement between LFT and SPT values, as well as between LFT plasma and serum results, was measured using the Cohen's kappa (κ) statistic. In the case of the concordance between LFT and SPT, expected agreement was also included as well as the Z statistic and the statistical significance. Calculations were per-

Table 2. LFT and SPT concordance

	SPT+ n	SPT- n	LFT value 1 considered positive					LFT value 1 considered negative				
			agree- ment, %	expected agreement, %	κ	Z	p	agree- ment, %	expected agreement, %	κ	Z	p
At least one positive wheal	180	90	90.7	52.7	0.81	13.4	<0.0001	84.1	50.5	0.68	11.7	<0.0001
Cat	48	222	91.1	73.1	0.69	11.1	<0.0001	91.8	74.6	0.68	11.5	<0.0001
Olive	62	208	90.7	62.0	0.76	12.5	<0.0001	90.0	64.8	0.72	11.7	<0.0001
<i>D. pteronyssinus</i>	123	147	88.5	50.4	0.77	12.6	<0.0001	85.2	50.9	0.70	11.7	<0.0001

formed by means of the statistical software STATA v. 7.0 (Stata Corporation, College Station, Tex., USA). As a rule of thumb, κ values between 0.61 and 0.80 are considered substantial, while those between 0.81 and 1.00 are considered almost perfect.

The aforementioned software package was also used to calculate the correlation between the intensity on the LFT line and the size of the SPT wheal (for each allergen analysed) by means of the Spearman's Rho. In addition, and again for each allergen, the mean wheal diameter (0 for a negative SPT result) was compared between the scores obtained in the LFT by means of ANOVA (Bonferroni procedure). Figures were drawn with SigmaPlot v. 9.0 software (Systat Software GmbH, Erkrath, Germany).

Ethical Approval

Phase II of the ISAAC study was approved by the ethics committee of the Doce de Octubre Hospital for all the Spanish centres involved in the study, including that of Cartagena.

Results

Agreement between Plasma and Serum Results in ImmunoCAP Rapid

Agreement between serum and plasma was almost perfect or perfect for cat ($\kappa = 0.93$), birch ($\kappa = 1.0$), mugwort ($\kappa = 0.87$), egg ($\kappa = 1.0$), olive ($\kappa = 0.92$) and *D. pteronyssinus* ($\kappa = 1.0$) and substantial for timothy grass ($\kappa = 0.71$), dog ($\kappa = 0.76$) and spreading pellitory ($\kappa = 0.78$). There were no positive results to cow's milk; therefore, the κ coefficient could not be calculated to this allergen.

Concordance between LFT and SPT Results

Data from the 270 plasma samples were analysed globally (at least 1 positive result in the SPT, independently of the allergen), and specifically for each allergen. Only results on cat, olive and *D. pteronyssinus* were analysed as these were the only ones with an acceptable number of positive individuals (according to SPT, the prevalence of sensitisation to birch, timothy grass and spreading pellitory in the selected children were 4.1, 7.4 and 3.0%, respectively).

The results of the concordance test are shown in table 2. Among the 270 samples, 180 showed at least 1 SPT-positive result; 123 were positive to *D. pteronyssinus*, 62 to olive and 48 to cat dander. Depending on value 1 of the LFT score (lightly visible LFT line) being considered as positive or negative, 2 groups of results are distinguished in the table (table 2).

When value 1 of LFT was considered positive, agreement and κ were generally higher as compared to the concordance tests obtained when value 1 was considered negative. The most important change occurred for 'at least 1 positive wheal' where agreement dropped from 90.7 to 84.1% and κ from 0.81 to 0.68.

Figure 1 shows the correlation (Spearman's Rho) between the mean wheal diameter in the SPT and the LFT score for each allergen. The correlation was quite high: 0.71 for cat, 0.81 for olive and 0.78 for *D. pteronyssinus*. This figure also depicts the mean wheal diameter of the SPT which corresponds to each value of the LFT score. Value 1 of this score corresponds to a wheal diameter lower than 3 mm for each and every allergen: cat 1.92 mm, olive 2.29 mm and *D. pteronyssinus* 2.76 mm.

Between each value of the LFT score, the change of the SPT mean wheal diameter was statistically significant in most cases. When it was not, it was probably due to the limited number of samples for that value. Between value 0 (LFT negative) and value 1 (lightly visible line) the increase in the wheal size was highly significant for the 3 allergens, as was also the case between values 1 and 3 (colour intensity as positive control) again for all allergens. The difference in the wheal size between values 1 and 2 (colour intensity lower than positive control) was only significant for cat, while between values 2 and 3 this was also so for olive and *D. pteronyssinus*. The difference between values 3 and 4 (colour intensity higher than positive control) was not significant for any allergen (fig. 1).

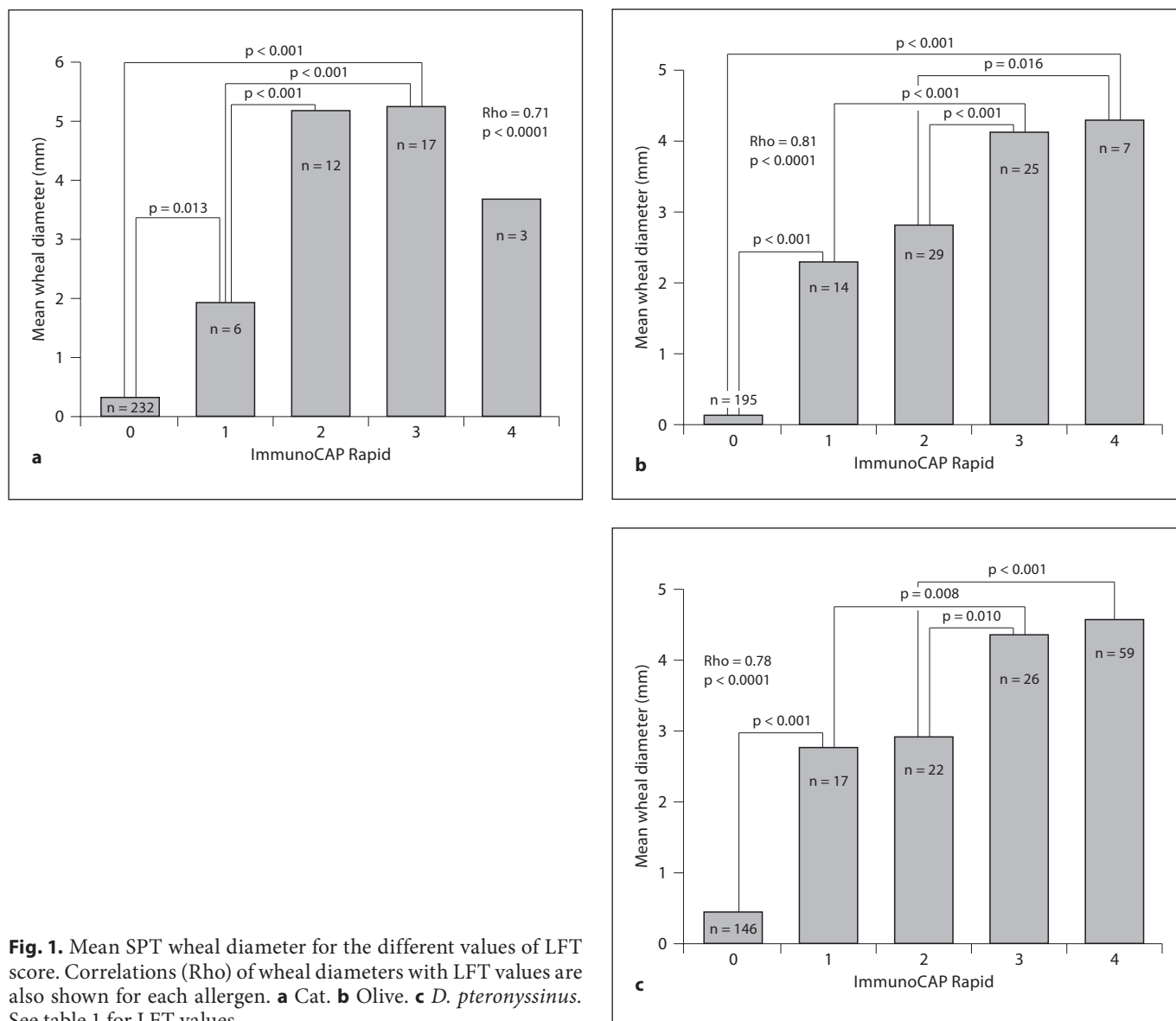


Fig. 1. Mean SPT wheal diameter for the different values of LFT score. Correlations (Rho) of wheal diameters with LFT values are also shown for each allergen. **a** Cat. **b** Olive. **c** *D. pteronyssinus*. See table 1 for LFT values.

Discussion

The results of the present study show that concordance between LFT and SPT is excellent and that the result of a 'lightly visible line' (value 1) should be considered positive, even though it corresponds to an SPT mean wheal diameter smaller than 3 mm: when this value is considered positive, κ is substantially higher (0.81 vs. 0.68). Moreover, the difference between the SPT mean wheal diameter corresponding to the value 0 in the LFT score and that for value 1 is the only adjacent step which is statistically significant for all allergens.

The high correlation between LFT score and SPT wheal diameter indicates that increases in SPT wheal size match with increases in the intensity of the colour line in the LFT. This can be seen graphically in figure 1, in which the SPT mean wheal diameter raises with the change from one value to another in the LFT score, that is, with the increase in the colour intensity of the line.

The main limitation of this study is that only 3 allergens could be evaluated. Only 6 allergens were common to SPT and LFT, and there were not enough samples for 3 of them. Nevertheless, there is no reason for the validity of the rest being different from the 3 allergens includ-

ed here. Moreover, each of these 3 allergens (cat, olive and *D. pteronyssinus*) represents a group of aeroallergens: epithelia, pollen and mite.

An additional limitation especially related to the lightly visible line is the fact that we used plasma instead of whole blood. Although in the manufacturer's opinion performance should be the same, it is possible that the lines were marked with slightly different intensities.

To the best of our knowledge, no prior study assessing LFT against SPT has been performed. There is 1 study that relates LFT with the amount of allergen-specific IgE measured by the ImmunoCAP system in a selected population (children 1–14 years of age with 3 or more wheezing episodes, with at least one of them within the previous year). The authors obtained values of κ between 0.74 and 0.84, depending on the definition of negative and positive values in the LFT [2].

Although there are no previous comparisons of LFT and SPT, there are studies which compare the reliability of the measurement of specific IgE in blood by the ImmunoCAP system in the laboratory as compared to the SPT, which are worthy of mention. Those comparisons have been made in general as well as in selected populations. Furthermore, within the selected populations there are studies carried out in patients simply referred to an allergy clinic, and also in patients who have been diagnosed as having an allergy. In the studies carried out in referred populations (with clinical symptoms of an allergic disease), the sample was generally about 100 patients. Agreement (referred to as accuracy or efficiency) for *D. pteronyssinus* ranged from 52 to 94% [5–7]. This high variability could be explained by the different criteria used to define a positive SPT. For instance, Nepper-Christensen et al. [5] used a wheal diameter larger than 5 mm, while Ewan and Coote [7] used a wheal diameter 2 mm larger than the negative one. In the studies performed in allergic individuals, the size of the sample population varied considerably, ranging from 49 patients to 357 serum samples (plus 50 control samples). Agreement obtained for *D. pteronyssinus* ranged from 76 to 87% [8–11]. With respect to studies in general populations, Gleeson et al. [12] included a sample of 167 children recruited at birth, in whom they carried out a prospective study. The agreement between SPT and ImmunoCAP was 93% for *D. pteronyssinus* and for cat.

The real usefulness of the LFT device is to be further clarified, but its potential seems high, not only for measuring sensitisation in a semi-quantitative way in the epidemiological field, as shown in the present paper, but also as an alternative to the ImmunoCAP system [2] – which requires a dedicated laboratory – in the allergy of-

fice. Its use should be, of course, put in the context of a clinical history. On the other hand, the device has the important limitation of offering – at least currently – a reduced panel of allergens.

Additionally, the analytical method incorporated by the LFT device may be useful for easy measurement of exposure to certain common allergens – such as dust mites – at home, if properly adapted, as it has been previously shown by Tsay et al. [13] and Polzius et al. [14]. This is an interesting field, important for the clinician and for the epidemiologist, which probably deserves more attention.

As for our results, when analysing the sensitisation to any allergen (at least 1 positive wheal), and when value 1 of LFT was considered positive, concordance is very high ($\kappa = 0.81$). However, when allergens are analysed individually, concordance drops, especially for cat. When LFT value 1 is considered negative, κ remains more similar for 'at least 1 positive wheal' and for each individual allergen, although the concordance is lower. In summary, LFT and SPT are comparable methods to identify sensitisation to cat, olive and *D. pteronyssinus* in the epidemiological field among schoolchildren.

Acknowledgements

This study has been sponsored by the Fondo de Investigación Sanitaria del Instituto de Salud Carlos III (PI06/90407). Phadia Diagnostics Spain supplied the kits needed to do the tests. We are grateful to Mr. Anthony Carlson for his help in correcting the English language of the paper.

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