

Defining thresholds of specific IgE levels to grass pollen and birch pollen allergens improves clinical interpretation



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ABSTRACT

Background: Cut-off values and predictive values are used for the clinical interpretation of specific IgE antibody results. However, cut-off levels are not well defined, and predictive values are dependent on the prevalence of disease. The objective of this study was to document clinically relevant diagnostic accuracy of specific IgE for inhalant allergens (grass pollen and birch pollen) based on test result interval-specific likelihood ratios. Likelihood ratios are independent of the prevalence and allow to provide diagnostic accuracy information for test result intervals.

Methods: In a prospective study we included consecutive adult patients presenting at an allergy clinic with complaints of rhinitis or rhinoconjunctivitis. The standard for diagnosis was a suggestive clinical history of grass or birch pollen allergy and a positive skin test. Specific IgE was determined with the ImmunoCAP Fluorescence Enzyme Immuno-Assay.

Results: We established specific IgE test result interval related likelihood ratios for clinical allergy to inhalant allergens (grass pollen, rPhl p 1,5, birch pollen, rBet v 1). The likelihood ratios for allergy increased with increasing specific IgE antibody levels. The likelihood ratio was <0.03 for specific IgE <0.1 kU/L, between 0.1 and 1.4 for specific IgE between 0.1 kU/L and 0.35 kU/L, between 1.4 and 4.2 for specific IgE between 0.35 kU/L and 3.5 kU/L, >6.3 for specific IgE >0.7, and very high (∞) for specific IgE >3.5 kU/L.

Conclusion: Test result interval specific likelihood ratios provide a useful tool for the interpretation of specific IgE test results for inhalant allergens.

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1. Introduction

Diagnosis of grass pollen and birch pollen allergy is based on patient history and the results of diagnostic tests for sensitization such as skin prick testing and allergen specific IgE. Skin tests and specific IgE provide proof for sensitization, but not necessarily for clinical disease. Provocation tests are considered the gold standard in assessing the link between the allergen and development of symptoms, but the feasibility of such test in a routine clinical setting or in large clinical studies is limited.

It is reasonable to assume that the likelihood for symptomatic grass and birch pollen allergic disease increases with increasing specific IgE antibody level. Several studies tried to determine diagnostic decision points in order to predict clinical reactivity [1–7]. For example, Schäfer et al. [3] reported that the positive predictive value increased with increasing specific IgE cut-off points for four common aeroallergens [grass and birch pollen, cat and *Dermatophagoides pteronyssinus*], whereas the negative predictive value decreased. It should be noted

that the predictive value is dependent on the prevalence of the disease. The prevalence of disease differs between geographical areas and does not always reflect the prevalence of disease in a particular clinical setting. Thus usefulness of reported predictive values for specific IgE tests is therefore limited.

Likelihood ratios allow to overcome this problem since they are independent of disease prevalence. The likelihood ratio is the ratio of the probability that a patient with the disease has a particular test result over the probability that a patient without the disease has the same test result. A likelihood ratio of 10, for example, means that a patient with the disease is 10 times more likely to have a certain test result than a patient without the disease. Besides, likelihood ratios can be calculated for various test result intervals. This makes it possible to document how the likelihood for disease increases with increasing antibody concentration.

In the present study, we examined whether specific IgE antibody level-dependent likelihood ratios against grass pollen and birch pollen extracts and against some components of these allergen extracts improve clinical interpretation. For that purpose, we performed a prospective study in which we included consecutive patients seen at an adult allergy clinic, because of respiratory upper airway complaints with suspected allergic cause. Our approach to evaluate

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the diagnostic accuracy was based on likelihood ratios, which is independent of prevalence, and allows to describe accuracy information for test result intervals [8,10].

2. Materials and methods

2.1. Study population

The study population consisted of 307 consecutive adult patients attending the Allergy Clinic for evaluation of suspected inhalation allergy and for whom clinical history and results of skin prick testing were available. In one patient with a clear history of severe birch pollen allergy and associated PR-10 related oral allergy syndrome, no skin testing was performed because of the high risk for side reactions. Sensitization to pollen demonstrated by skin testing was considered symptomatic when at least one of the following symptoms: rhinorrhea, conjunctivitis, sneezing or nasal obstruction was present, recurred or exacerbated yearly during the grass or tree pollen season and/or exacerbated upon outdoor exposure to pollen. Patients with typical clinical history and symptoms and positive skin prick test were considered allergic, while patients without the typical history and symptomatology and with negative skin prick tests served as diseased controls. Some patients ($n = 84$) were sensitized for both birch and grass pollen.

The study was approved by the local ethical committee and patients gave an informed consent before inclusion in the study.

2.2. Skin prick tests

Patients underwent skin prick testing with commercial allergen preparations from HAL Allergy, Leiden, The Netherlands (grass and birch pollen). Tests were performed and interpreted according to the guidelines of the European Academy of Allergology and Clinical Immunology [9]. A test with a wheal diameter of >3 mm above the control wheal is considered positive.

2.3. Allergen-specific IgE antibodies

Specific IgE was measured using CAP-FEIA technology (Thermo Fisher, Uppsala, Sweden) on a Unicap 1000 instrument according to the instructions of the manufacturer. The detection limit was 0.1 kU/L IgE. Specific IgE antibodies were determined against two inhalant allergen extracts [grass pollen mixture (*Anthoxanthum odoratum*, *Lolium perenne*, *Phleum pratense*, *Secale cereale*, *Holcus lanatus*) and birch pollen] and against recombinant components of *P. pratense* and *Betula verrucosa* (rPhl p 1.5 and rBet v 1).

2.4. Statistical analysis

Likelihood ratios were calculated as described previously [10]. The likelihood ratio gives an estimation of whether there will be a significant change in pre-test to post-test probability of disease given the test result [8,10]. A likelihood ratio of 1 implies that there will be no difference between pre-test and post-test probabilities [8]. Likelihood ratios >10 or <0.1 indicate large, often clinically significant differences in most circumstances. Likelihood ratios between 5 and 10 and between 0.1 and 0.2 indicate modest clinical differences, likelihood ratios between 2 and 5 and between 0.5 and 0.2 indicate small but potentially relevant clinical differences, likelihood ratios between 1 and 2 and between 0.5 and 1 indicate small differences (rarely clinically significant).

Using likelihood ratios, one can calculate the post-test probability for any given pretest probability [8,10].

3. Results

3.1. Population characteristics

Population characteristics are summarized in Table 1. There was a good agreement between clinical history and skin prick testing (97.5–98.5% for grass pollen and 95–100% for birch pollen). For grass pollen, 4 subjects had a positive skin prick test but no suggestive history, while only 2 of the subjects with a suggestive history for grass pollen allergy had a negative skin prick test. For birch pollen, 9 subjects with a negative history had a positive skin prick test, while none of the subjects with a suggestive history for birch pollen allergy had a negative skin prick test.

Among the total patient group, 135 (61 males and 74 females) and 123 (49 males and 74 females) had grass or tree pollen allergy, respectively. Diseased controls were the 161 patients (47 males and 114 females) negative for grass pollen and 173 patients (56 males and 117 females) negative for birch pollen (Table 1). The 15 with discordance between history and skin tests were not included in the calculation of likelihood ratios. Calculation of likelihood ratios is based on a comparison of results in a population with definite diagnosis of disease (in this case positive clinical history and positive skin prick test) and a population with no disease (in this case negative clinical history and negative skin prick test).

3.2. Likelihood ratios

We calculated the likelihoods for specific IgE results for different test result intervals [<0.1 kU/L, 0.1 kU/L–0.35 kU/L, 0.35 kU/L–0.7 kU/L, 0.7–3.5 kU/L, 3.5 kU/L–17.5 kU/L, 17.5–50 kU/L, 50 kU/L–100 kU/L, and >100 kU/L] for subjects with definite allergy (patients) and for subjects with no pollen allergy (controls). For each test result interval, the likelihood ratio (i.e. the likelihood in those with allergy divided by the likelihood in those without pollen allergy) was calculated. Fig. 1 and Table 2 show the test-result interval-specific likelihood ratios as well as the likelihoods (Fig. 1) (i.e. fraction of patients or controls with a particular test result) for allergen specific IgE to natural extracts (grass pollen extract), birch pollen or allergen components (rPhl p 1.5, rBet v 1).

Our data show that the likelihood ratios increase with increasing specific IgE antibody levels. For all allergens tested, the likelihood ratio of a specific IgE value >3.5 kU/L was very high (infinite) (no such high values were found in controls). Sixty-nine (for rPhl p 1.5) to 72% (for extract) of grass pollen allergic patients had such high values, whereas 78% (rBet v 1) to 82% (extract) of birch pollen allergic patients had such high values.

The likelihood ratio of a specific IgE value between 0.7 kU/L and 3.5 kU/L (found in 15% of birch pollen allergic patients, in 21–23% of grass pollen allergic patients, and in $\leq 3\%$ of controls) was very high (infinite) for the allergen components (rPhl p 1.5, rBet v 1) and was infinite for grass pollen mixture and 6.3 for birch pollen extract.

A test result between 0.35 kU/L and 0.7 kU/L (found in 1.6–4.0% of the patients and in 0.6–3.1% of the controls) had a likelihood ratio that varied between 1.2 and 4.2, indicating no or a small difference in pre- to post-test probability. A test result between 0.1 kU/L and 0.35 kU/L (found in 2–3.3% of the patients and in 2.3–9.9% of the controls) had a

Table 1

Number of grass pollen and birch pollen patients according to the clinical history and the results of skin prick testing.

Clinical history	+	+	–	–
Skin prick testing	+	–	+	–
Grass pollen	135	2	4	161
Birch pollen	123	0	9	173
	Allergic	Possible allergy	Sensitization	Non-allergic

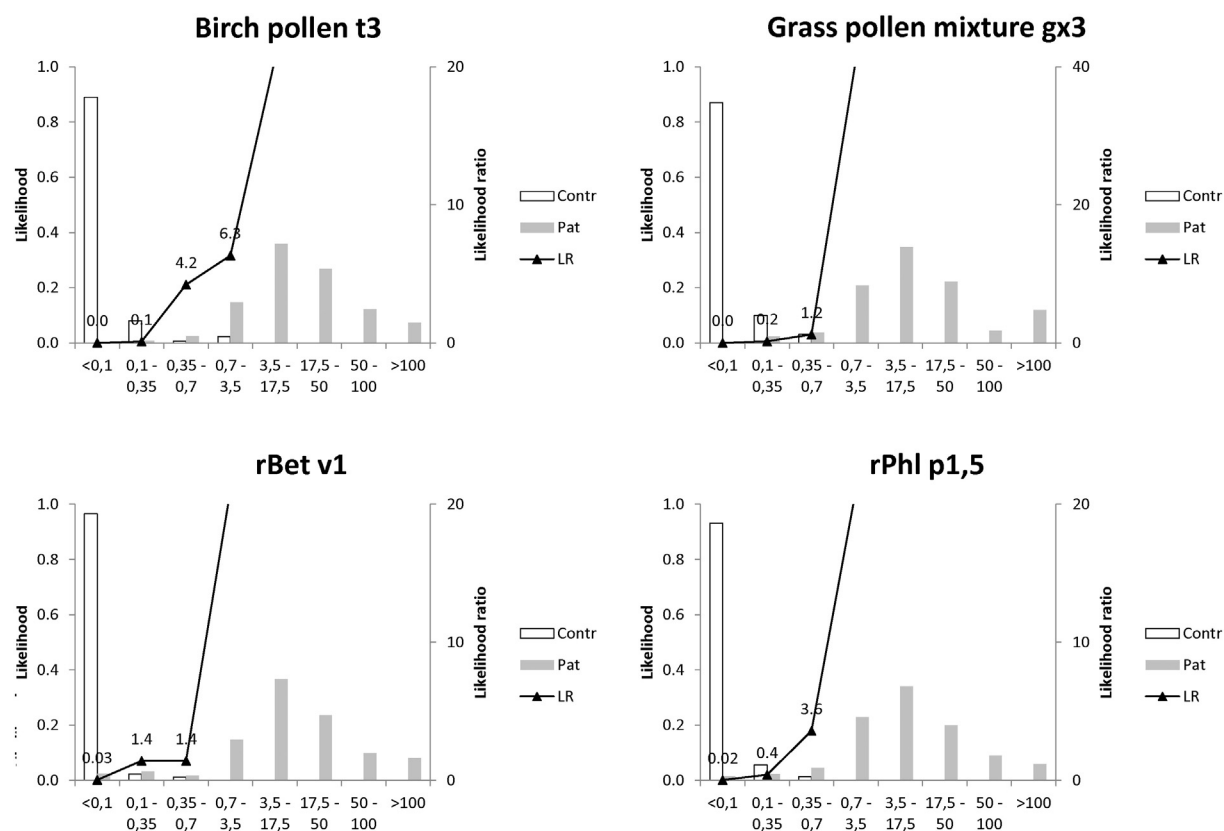


Fig. 1. Likelihoods (left y-axis) of anti-IgE antibody test results [<0.1 kU/L, between 0.1 and 0.35 kU/L, between 0.35 and 0.7 kU/L, between 0.7 and 3.5 kU/L, between 3.5 and 17.5 kU/L, between 17.5 and 50 kU/L and >100 kU/L] for grass pollen mix (GP), rPhl p 1,5, birch pollen (BP) and rBet v 1 for rhinitis controls (open bars) and grass pollen allergic patients (filled bars). The triangles represent the likelihood ratios (second y-axis).

likelihood ratio of 0.1–1.4, indicating a modest or no difference in pre-test to post-test probability.

IgE values <0.1 kU/L (found in 87%–96% of the controls and 0–2.4% of the patients) had a likelihood ratio of <0.03 , indicating a clinical significant difference in pre-test to post-test probability. Of particular interest, the likelihood ratio was 0 for the extracts (meaning that none of the grass pollen or birch pollen allergic patients had a sIgE <0.1 kU/L with the extracts), whereas it was 0.02–0.03 for the components (meaning that a small fraction of grass pollen allergic patients [1%] or birch pollen allergic patients [2.4%] did not have sIgE to rPhl p 1,5 or rBet v 1, respectively).

Fig. 2 shows the post-test probability for birch and grass pollen allergy as a function of the pre-test probability and of the specific IgE antibody level. On the graph one can easily read the post-test probability for allergy for any given pre-test probability and specific IgE test result. For example, for a pre-test probability of 9.7% [which corresponds to the prevalence of symptomatic sensitization to trees in Belgium [11]], the post-test probability for symptomatic allergy to tree pollen is 0%, 1%,

31%, 40%, and 100%, for a specific IgE to birch pollen of <0.1 kU/L, between 0.1 and 0.35 kU/L, between 0.35 and 0.7 kU/L, between 0.7 and 3.5 kU/L, or >3.5 kU/L, respectively. The results are summarized in Table 3. Table 3 also illustrates the post-test probabilities for a pre-test probability of 44%, which corresponds to the prevalence of birch pollen allergy in a population with rhino-conjunctivitis attending a specialized allergy clinic (this study). Additionally, the table illustrates the post-test probabilities for symptomatic grass pollen allergy for a pre-test probability of 17.6% (which corresponds to the symptomatic sensitization to grass pollen in Belgium [11] and 44% [which corresponds to the prevalence of grass pollen allergy in a population with rhino-conjunctivitis attending a specialized allergy clinic (this study)]).

4. Discussion

In this study we established specific IgE test result interval related likelihood ratios for clinically relevant allergic sensitisation to two inhalant allergens (grass and birch pollen). We evaluated the IgE antibody

Table 2

Likelihood ratios (95% confidence intervals) for allergy to grass pollen and birch pollen for different specific IgE test result intervals. GP: grass pollen mix; BP: birch pollen. ∞: infinity.

Allergen	sIgE (kU/L)				
	<0.1	0.1–0.35	0.35–0.7	0.7–3.5	>3.5 –17.5
Grass pollen mixture	0.00	0.22 (0.07–0.75)	1.19 (0.35–4.03)	∞	∞
rPhl p 1,5	0.02 (0.004–0.06)	0.4 (0.11–1.44)	3.6 (0.73–17.44)	∞	∞
Birch pollen	0.00	0.1 (0.01–0.75)	4.22 (0.44–40.09)	6.33 (2.19–18.24)	∞
rBet v 1	0.03 (0.01–0.08)	1.41 (0.36–5.52)	1.41 (0.20–9.85)	∞	∞

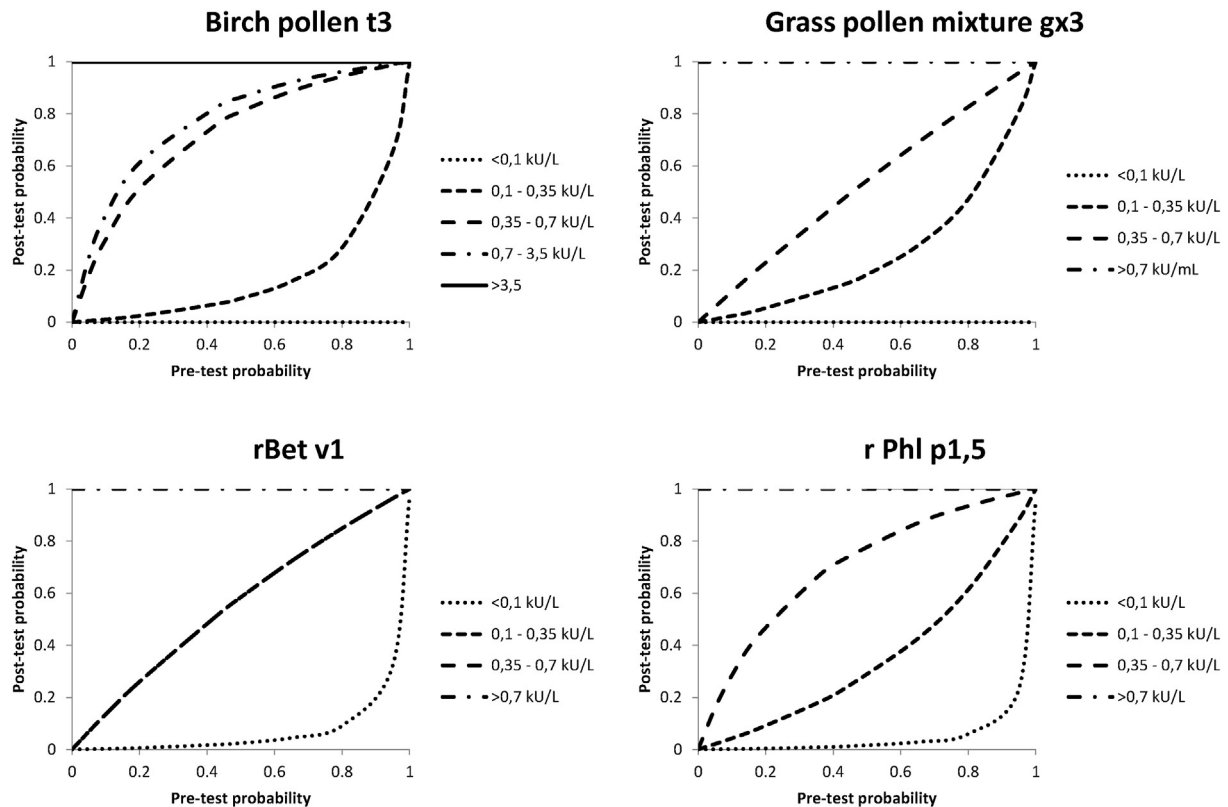


Fig. 2. Post-test probability for reactivity to inhalant allergens as a function of pre-test probability and of specific IgE test result [<0.1 kU/L, between 0.1 and 0.35 kU/L, between 0.35 and 0.7 kU/L, between 0.7 and 3.5 kU/L, between 3.5 and 17.5 kU/L, and >3.5 kU/L] for grass pollen mix (GP), rPhl p 1,5, birch pollen (BP), and rBet v 1.

assay for grass and birch pollen extracts and for the major allergen components Phl p 1,5 and bet v 1 in consecutive patients with a clear clinical history of pollen allergy and in disease controls (i.e. subjects with upper respiratory complaints attending an allergy clinic, but no suggestive history for pollen allergy and negative skin tests).

Phl p 1,5 represents the most important allergen components among grass pollen allergic subjects, with IgE-binding frequencies exceeding 80% [12], and useful as markers for genuine sensitization to grass pollen [13,14]. Bet v 1 is the major birch pollen allergen detecting $>95\%$ of the sera positive for IgE to natural birch pollen extract [15]. Reactivity to Bet v 1 indicates sensitization to tree pollen of the order Fagales [13].

In addition to patient history, diagnostic tests are crucial in the diagnosis of allergic disease. Skin testing is the most commonly used diagnostic method for sensitization, but specific IgE testing is progressively becoming more popular, especially for general practitioners not having the time and infrastructure for skin testing. It is generally accepted that higher levels of allergen-specific IgE are associated with increased likelihood for allergic reactions. For aeroallergens only few data concerning the dose-relationship between specific IgE levels and clinical reactivity exist. Pastorello et al. [1] studied cut-off values for specific IgE levels for seasonal aeroallergens (house dust mite, cat and dog) that could discriminate between symptomatic and asymptomatic patients. Most studies, however, focused on food allergy and food allergen-specific IgE levels as predictors for risk [2,4–7]. As already mentioned by Ballmer-Weber [16], these predictive values lack precision, since they are very different between different populations, indicating that such decision points are not very helpful for diagnosis. Indeed, predictive values are dependent on disease prevalence, which may differ between geographical areas and between studies and clinical practice. Therefore, we here propose likelihood ratios to document the diagnostic accuracy of specific IgE determinations. Likelihood ratios have the advantage that they are independent of the disease prevalence. In addition to

providing likelihood ratios we also provided a visual representation of how the post-test probability of clinically relevant allergic sensitization depends on the pre-test probability and the level of specific IgE. Such graphical representation when added to a test result can easily be used by practitioners.

We have previously shown that the use of likelihood ratios and a graphical display of post-test probability in the function of pre-test probability and antibody level improves the clinical interpretation of anti-CCP antibodies for diagnosis of rheumatoid arthritis [17] and of enzyme immunoassays for the diagnosis of small-vessel vasculitis [18] and celiac disease [19].

Table 3

Post-test probability for allergy to birch pollen or grass pollen as a function of defined pre-test probabilities.

The prevalence of symptomatic sensitization to trees and to grass pollen is 9.7% and 17.6%, respectively [11]. The prevalence of birch pollen allergy and of grass pollen allergy in a population with rhinoconjunctivitis attending a specialized allergy outpatient clinic is $\pm 43\%$ (this study).

GP: grass pollen mix, BP: birch pollen.

Pre-test probability	Allergen	Post-test probability				
		<0.1 kU/L	0.1–0.35 kU/L	0.35–0.7 kU/L	0.7–3.5 kU/L	>3.5 kU/L
<i>Grass pollen allergy</i>						
17.6%	GP	0%	5%	20%	100%	100%
	rPhl p 1,5	0.3%	8%	43%	100%	100%
44%	GP	0%	15%	48%	100%	100%
	rPhl p 1,5	1%	24%	74%	100%	100%
<i>Birch pollen allergy</i>						
9.7%	BP	0%	1%	31%	40%	100%
	rBet v 1	0.3%	13%	13%	100%	100%
40%	BP	0%	6%	74%	81%	100%
	rBet v 1	1.6%	48%	48%	100%	100%

Although most clinical studies use 0.35 kU/L as the cut-off value for clinical reactivity [14,20,21], our data show that higher values provide more strong diagnostic accuracy, as likelihood ratios increase with increasing specific IgE antibody levels. Values exceeding 3.5 kU/L strongly suggest clinically relevant allergy, whereas values between 0.7 and 3.5 kU/L also significantly impact post-test probability, with very high likelihood ratios for the components and a likelihood ratio of 6.3 for birch pollen extract and infinity for grass pollen extract. Values between 0.35 and 0.7 kU/L have low likelihood ratios (between 1.4 and 3.6).

Importantly, quantifying specific IgE down to 0.1 kU/L a test result of <0.1 kU/L has a likelihood ratio <0.1, indicating a clinically significant decrease in pre-test to post-test probability, thus excluding allergy, whereas a test result between 0.1 kU/L and 0.35 kU/L has likelihood ratios that vary between 0.1 and 1.2, indicating only a modest or no (rBet v 1) change in pre-test to post-test probability. In other words, a test result of <0.1 kU/L more strongly excludes allergy than a test result between 0.1 kU/L and 0.35 kU/L. Of special interest, the likelihood ratio of a IgE value <0.1 kU/L was 0 for both extracts tested, but not for the recombinant allergens, indicating that the recombinant allergen components may fail to detect allergy in some (1–2%) patients.

Double positivity for skin prick testing and clinical history was used as reference. This approach might introduce a small bias as some rare allergic patients that test negative by skin prick testing might not be included. Likelihood ratios calculated based on clinical history as reference were not significantly different from likelihood ratios calculated based on clinical and skin prick testing as referenced (data not shown).

The likelihood ratios derived in this study hold for a Belgian adult population. Additional larger studies, that also include children, are needed to validate the results and to evaluate whether there are differences between adults and children. There might also be geographical differences. For example, it should be evaluated whether likelihood ratios for birch pollen are different between Southern Europe and Belgium.

Recently, Hamilton [22] proposed that clinical laboratories should provide analytical measurements and let the physician decide whether the results are clinically relevant. We however, argue that it is a shared responsibility of laboratory professionals and specialists in allergy to help with the interpretation of a specific IgE test result. Such added-value strategy [23] can be realized by providing test result-specific likelihood ratios. This might need further education, as most physicians are not familiar with such approach.

In conclusion, we illustrated how test result interval specific likelihood ratios may help with the interpretation of specific IgE test results. Clinical laboratories might consider to provide allergen-specific and test result interval specific likelihood ratios. As an alternative, a graphical representation of the allergen-specific post-test probability as a function of the pre-test probability and the antibody level can be provided to the clinicians. Such information may help with the interpretation of a specific IgE antibody test result.

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