

Can serum white birch (*Betula verrucosa*) pollen antigen (Bet v 1) immunoglobulin E measurement distinguish between sensitization and allergy?

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Background: In clinical practice, it is fundamental to distinguish between sensitization and allergy, mainly with respect to polysensitized patients. The aim of this study was to evaluate whether serum immunoglobulin E (IgE) to white birch (*Betula verrucosa*) pollen antigen (Bet v 1) assessment might be able to differentiate simple sensitization from true allergy.

Methods: In total, 197 subjects (100 females; mean age 44.9 years) with serum Bet v 1 IgE positivity and history of nasal symptoms were evaluated. Subjects were subdivided in 2 groups: allergic and sensitized.

Results: Allergic patients were 58.4% of the total; they had higher serum Bet v 1 IgE levels than sensitized subjects ($p < 0.001$). A serum IgE to Bet v 1 value >8.94 kilo units of allergen per liter (kUA/L) was able to differentiate between sensitization and allergy (area under the receiver operating characteristic curve [AUC] = 0.76; diagnostic odds

ratio, ie, degree of agreement between health status and dichotomized IgE levels [DOR] = 6.5). Gender and season when the blood was drawn influenced IgE levels.

Conclusion: Serum Bet v 1 IgE measurement may be able to differentiate sensitization from allergy in subjects with sensitization to *Betulaceae* pollen allergens. Thus, Bet v 1 IgE measurement should be considered a useful step in the management of patients with allergic rhinitis due to *Betulaceae* pollen allergens. © 2015 ARS-AAOA, LLC.

Key Words:

allergic rhinitis; serum IgE; Bet v 1; sensitization; allergy

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Allergic rhinitis (AR) is a common immunoglobulin E (IgE)-mediated inflammatory disorder.^{1,2} IgE may be considered the hallmark of allergy. The formal diagnosis of true allergy is based on consistency between simple sensitization, such as IgE production documented by cutaneous testing and/or serum assay, and symptoms history.³ In other words, sensitization is not synonymous with allergy: a sub-

ject may be only sensitized and not allergic, but a patient with allergies should necessarily be sensitized. Moreover, polysensitization, or sensitization to 2 or more allergens, is very common: it occurs in up to 80% of patients with allergy.^{4,5} This phenomenon may have clinical implications, especially in the matter of allergen immunotherapy (AIT), even though it has been shown that AIT could also be effective in polysensitized patients.⁶ Therefore, it is mandatory to distinguish sensitization from allergy, mainly in polysensitized patients. However, it is not always easy to differentiate sensitization from allergy; for example, the pollination periods of different pollen species may frequently overlap. To respond to this problem, 2 previous studies showed that serum IgE assessment was more useful than skin-prick testing (SPT) in distinguishing between sensitization and allergy.^{7,8} In addition, it has been reported that *Arachis hypogaea* (peanut) (Ara h 2)-specific and *Corylus avellana* (hazelnut) (Cor a 14)-specific IgE are useful to predict the positive response to challenge.^{9,10}

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Both tests are standardized and the outcomes are quite consistent between the 2 tests, even though discrepancy may occasionally exist.¹¹ Serum IgE assessment allows a standardized quantitative measurement. In addition, molecular-based allergy diagnostics allows us to precisely define and to characterize the sensitization profile, helping with the AIT prescription, because positivity to major allergens excludes false reactivity to pan-allergens.^{12,13} Birch allergy is common in our area.¹⁴ Bet v 1 is the genuine and major molecule of *Betulaceae* family allergens. Therefore, this study evaluated whether serum Bet v 1 IgE assessment could differentiate between sensitization and allergy, also considering age, gender, and seasonality of blood draw.

Patients and methods

This retrospective study considered 197 patients (100 females; mean age 44.9 years) who were referred to the Allergy Department because of a suspected allergy. Inclusion criteria were serum IgE positivity to Bet v 1, at >0.35 kilo units of allergy per liter (kUA/L), and history of nasal symptoms, including itching, sneezing, watery rhinorrhea, and nasal obstruction. The study was performed according to the Review Board rules.

Patients were subdivided in 2 groups on a clinical ground: sensitized subjects (without symptoms after natural exposure to *Betulaceae* pollen) and allergic patients (with symptoms occurring after *Betulaceae* pollen inhalation during its pollen season). *Betulaceae* pollens consist of different species, including birch, hazel, alder, hornbeam, and hop-hornbeam. The *Betulaceae* pollen season usually lasts from February to April in our geographic area, as documented by previous studies based on specific pollen counts.¹⁵ Patients were treated with symptomatic drugs only on demand.

The diagnosis of true birch allergy was performed exclusively by well-trained allergists (G.C., P.M., S.V., D.B., and C.T.) asking about the presence of symptoms during the period February to April, which is particularly peculiar for *Betulaceae* pollens in our area.

Serum IgE assay

Serum levels of specific IgE were detected by immunofluorometric assay (IFMA) (ImmunoCAP; Thermo Fisher Scientific, Uppsala, Sweden) in peripheral blood samples from patients. Serum was collected into gel-separator tubes, centrifuged, and stored at -20°C until analysis for Bet v 1.

Measurement of circulating specific IgE antibodies was performed according to manufacturer's instructions.¹⁶ Specific IgE concentrations were expressed in kUA/L according to the traceable calibration to the 2nd World Health Organization (WHO) International Reference Preparation (IRP) for Human IgE and 0.35 kUA/L has been considered as a cutoff.¹⁷

Statistical analysis

Distributions of IgE levels by gender, age, immunological status (ie, sensitization or allergy) and time at draw blood, were graphically explored using histograms, box plots, and Q-Q plots. Given the positive skewness of IgE levels, data were log-transformed and described using geometric mean (GM), geometric standard deviation (GSD), and interquartile range (IQR).

The relationships between patients' characteristics and IgE measurements were assessed through multiple linear regression modeling.¹⁸ In this framework, age was categorized into seven 20-year groups and modeled as a set of dummy variables. Also, month at draw blood was used as an indicator of seasonality. Log-transformation was applied to IgE levels to meet the Gaussian assumptions of regression modeling.¹⁹ After the fitting process, parameter estimates were antilog-transformed in order to improve interpretability of regression results by restoring the original IgE scale of measurement (kUA/L). In addition, such a back-transformation amounted to assuming the ratio between GMs as an index of association between the observed IgE levels and explanatory factors entered the regression equation. In all modeling statistical significance was assessed via an F-test.

The classification performance of the study biomarkers was addressed using receiver operating characteristic (ROC) curve analysis. In particular, the area under the ROC curve (AUC) was used as an estimate of the overall correct classification (accuracy) of enrolled patients, and the Youden index (YI) as a statistical criterion to define optimal classification cut-points (OCCs), namely allergen-specific IgE values capable of minimizing false classification rates.²⁰ According to the OCCs and given a target health condition (sensitized or allergic), the following classification parameters were also estimated: sensitivity (Se) and specificity (Sp) (ie, proportion of positives/negatives among patients with/without the target condition); diagnostic odds ratio (DOR) (ie, the degree of agreement between health status and dichotomized IgE levels); positive predictive value (PPV) and negative predictive value (PNV) (ie, the proportion of patients with/without the target condition among positives/negatives).

The statistical significance of each AUC was evaluated using the Wilcoxon-Mann-Whitney test.

In all contexts, statistical indexes were provided with 95% confidence limits (95% CLs) and a p value <0.05 was considered as statistically significant.

All data were analyzed using the Stata statistical package, Release 13.1 Statistical Software. (StataCorp, College Station, TX).

Results

Overall, 197 subjects were enrolled in this investigation. Females were older than males (42.2 vs 35.4 years) and the overall IQR for age was 20.1 to 77.4 years.

TABLE 1. Distribution of IgE-specific levels by immunologic status

IgE	Immunologic status	Patients		GM	GSD	IQR
		n	%			
Bet v 1	Sensitization	82	41.6	2.2	6.7	0.8–7.0
	Allergy	115	58.4	10.3	2.2	4.1–26.4
	All	197	100.0	5.4	5.6	2.2–18.6

Bet v 1 = white birch (*Betula verrucosa*) pollen antigen; GM = geometric mean; GSD = geometric standard deviation; IgE = immunoglobulin E; IQR = interquartile range.

TABLE 2. Joint effect of age, gender, immunological status, and seasonality on log-transformed allergen-specific IgE levels estimated using multiple linear regression modeling

Factors and levels	Bet v 1		
	GM	95% CL	<i>p</i> ^a
Immunological status			<0.001
Sensitization	3.2	1.5–6.4	
Allergy	13.0	6.0–27.5	
Seasonality			<0.001
Winter	3.2	1.5–6.4	
Spring	6.4	3.4–11.9	
Summer	5.1	2.4–10.7	
Autumn	2.0	1.0–3.8	
Gender			0.003
Male	3.2	1.5–6.4	
Female	1.7	0.7–3.4	
Age			0.578
≤20 years	3.2	1.5–6.4	
21–30 years	2.4	1.1–4.7	
31–40 years	2.7	1.3–5.6	
41–50 years	1.9	0.8–4.0	
51–60 years	3.3	1.6–6.9	
>60 years	2.4	0.9–5.7	

^a*p* value represents the significance level of the F-test.

Bet v 1 = white birch (*Betula verrucosa*) pollen antigen; CL = confidence limits of GM; GM = geometric mean; IgE = immunoglobulin E.

Table 1 shows some results of exploratory analyses. One hundred and fifteen subjects (58.4%) were allergic to *Betula* pollens. It is worth noting that allergic patients had higher levels of Bet v 1 than sensitized patients. There was no patient negative to Bet v 1 IgE and still allergic to birch.

Table 2 reports the joint effects of age, gender, immunological status (such as sensitization or allergy), and

TABLE 3. Results of ROC curve analysis performed on the study serum-specific IgE levels*

Classification parameter	Bet v 1	
	Estimate	95% CL
Area under ROC curve	0.76	0.69–0.83
Youden index	0.41	0.06 ^a
Optimal classification cut-point	≥8.94	3.1–14.7
Sensitivity	0.59	0.49–0.68
Specificity	0.82	0.71–0.89
Diagnostic odds ratio	6.5	3.3–12.6
Prevalence ^b	0.58	0.51–0.65
Positive predictive value	0.82	0.72–0.89
Negative predictive value	0.59	0.49–0.67

*Allergic status is assumed to be the target immunological condition.

^aStandard error of the Youden index.

^bProportion of allergic patients.

Bet v 1 = white birch (*Betula verrucosa*) pollen antigen; CL = confidence limits; IgE = immunoglobulin E; ROC = receiver operating characteristic.

seasonality on the study biomarker, estimated through the multiple regression analysis. Immunological status played a significant role with respect to Bet v 1-specific IgE level. In particular, Bet v 1 IgE GM in allergic patients was found to be about 4.1 times higher than that of sensitized patients (95% CL, 2.6 to 6.4; *p* value <0.001). A clear seasonal trend was highlighted (Fig. 1) with maximum/minimum smoothed values of 7.7/2.6 in May/December, obtained through locally weighted scatterplot smoothing (LOWESS) fitting.¹⁶ Moreover, Bet v 1 IgE GM levels in females were about 50% lower than those obtained in males (GM ratio = 0.52; 95% CL, 0.34 to 0.80). Finally, age seemed to give no remarkable contribution to explain the observed variability of any specific IgE level.

Table 3 shows the results of the ROC analysis. In order to estimate classification parameters, allergy was assumed as the target immunologic condition in all analyses. In this setting, the ROC methodology pointed out a high overall accuracy for Bet v 1 IgE (AUC = 0.76; 95% CL, 0.69 to 0.83; Fig. 2)

On the basis of YI, the following OCC value was estimated: 8.94 kUA/L (Table 3). Given that allergic patients had a higher GM value for Bet v 1 IgE (Table 2), allergy could be defined when Bet v 1 IgE ≥8.94. In this context, the capability of correctly classifying allergic subjects was lower than 0.70 (Se = 0.59; 95% CL, 0.49 to 0.68). On the other hand, under the same conditions, it showed very high performance with respect to correctly classifying sensitized individuals (Sp = 0.82; 95% CL, 0.71 to 0.89).

DOR estimates confirmed the remarkable role of Bet v 1 IgE in the classification process. In fact, the probability of being allergic among Bet v 1-positive subjects was about

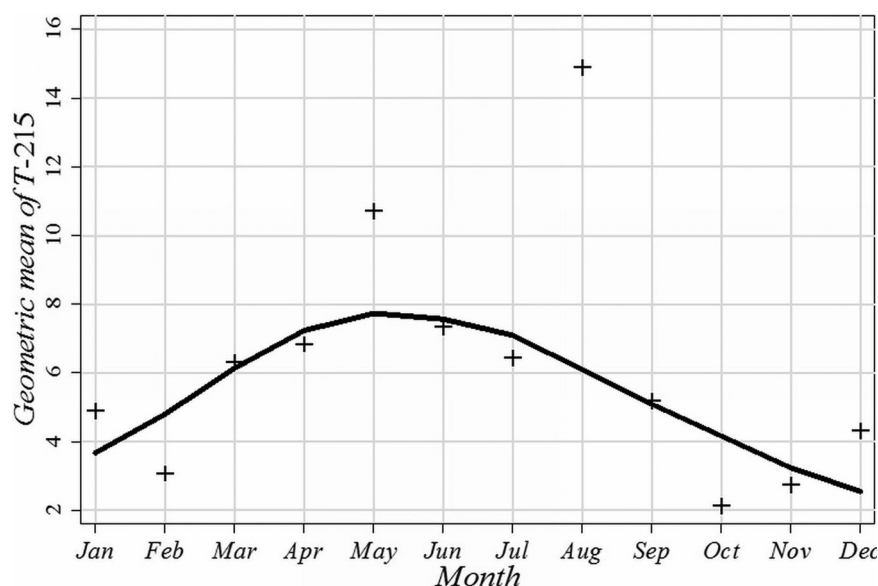


FIGURE 1. Seasonal trend of Bet v 1 IgE geometric mean level estimated through linear regression modeling adjusted for immunological status, gender, and age. Bet v 1 IgE = white birch (*Betula verrucosa*) pollen antigen immunoglobulin E

6 times higher than the same probability among Bet v 1-negative ones (DOR = 6.5; 95% CL, 3.3 to 12.6).

In addition, a very high probability of being allergic among IgE-positive patients was estimated (PPV = 0.82; 95% CL, 0.72 to 0.89). Finally, regarding the distribution of sensitized patients among negatives, returned NPV was statistically less than 0.50 (Table 3).

Discussion

AR diagnosis is based on consistency between exposure to sensitizing allergen and resulting symptom occurrence.^{21,22}

The current study showed that more than one-half of the considered subjects were allergic to *Betulaceae* pollens, confirming the concept that not all sensitized subjects are really allergic. In effect, AR patients had higher serum Bet v 1 IgE levels than sensitized subjects. A reliable cutoff able to predict *Betulaceae* allergy was defined: a value >8.94 kUA/L had a DOR of 6.5. Therefore, serum specific-IgE measurement may significantly improve the diagnostic capability of the clinician. On the other hand, there were a few patients (8 patients) with lower expression of Bet v 1, such as <8.9 kUA/L and still allergic: it might depend on allergy to other epitopes. In addition, this study showed that males had higher IgE levels than females. This finding could depend on different hormonal patterns, which could affect the immune response. In fact, the present findings confirmed previous epidemiological surveys showing higher (both total and specific) IgE levels in males.^{23–27} Contrastingly, age did not significantly influence the Bet v 1 IgE levels.

Interestingly, the blood draw season could impact on Bet v 1 IgE levels with a peak between April and June. This phenomenon could depend on direct exposure to pollen in the air: *Betulaceae* pollen season usually lasts from

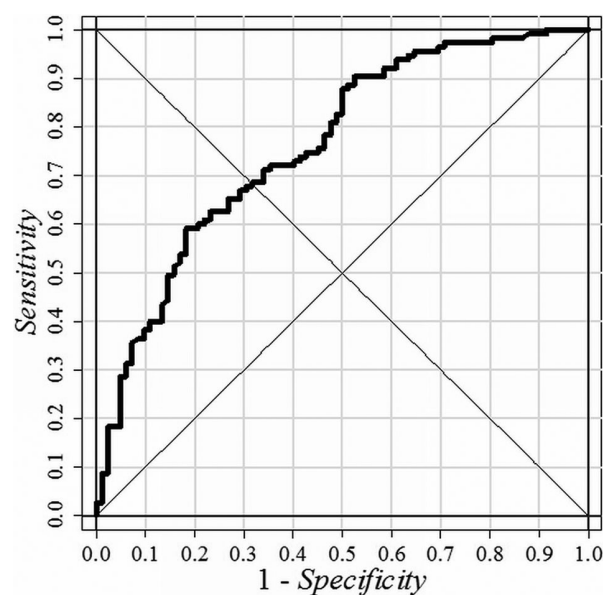


FIGURE 2. ROC curve for immunological status based on Bet v 1 IgE. Bet v 1 IgE = white birch (*Betula verrucosa*) pollen antigen immunoglobulin E; ROC = receiver operating characteristic.

February to April. Therefore, the airborne pollen presence could influence allergen-specific IgE production, as was reported for both pollens and mites.^{27–31}

On the other hand, a main limitation of this study is that it was cross-sectional. This aspect opens interesting future lines of research, including speculation about what will happen with sensitized patients in the future: will some transform into allergic? However, the opposite question is: could allergic patients develop tolerance and become only sensitized? In this regard, AIT could modify the natural history because it is able to induce immunological and clinical tolerance toward the used allergen extract, but a

spontaneous tolerance might happen too. As many questions remain unmet, the future research should address these topics, including the effect of season on IgE levels and the long-term follow-up of both sensitized and allergic patients. In addition, another crucial point is to prove that allergic patients maintain their statistically elevated IgE levels compared to sensitized patients year-round. Also, a relevant drawback of this study is that all serum IgE the findings of different laboratories are not equal and this threshold would have to be defined for each laboratory test. Moreover, it has to be considered that the sure demonstration of allergy is obtained by allergen-specific challenge, but this procedure is time-consuming and expensive, so it is often omitted in the allergy workup. An alternative way

for future studies should be to measure symptoms by a symptom-scale, as the allergen-specific challenge was not performed, still it is possible that the patients could react to other allergens during the same period and not only to the considered antigen.

Conclusion

Bet v 1 IgE measurement may be able to differentiate sensitization from allergy in subjects sensitized to *Betulaceae* pollens. Thus, IgE measurement should be considered a useful step in the management of patients with AR due to *Betulaceae* pollen allergens. ③

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