

# Effects of Airborne Birch Pollen Levels on Clinical Symptoms of Seasonal Allergic Rhinoconjunctivitis

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## Key Words

Panel study · Nonlinear effects, pollens · Birch · Seasonal allergic rhinitis · Threshold · Plateau · Aerobiology · Air pollution · Hay fever

## Abstract

**Background:** Most panel studies focusing on rhinitis and conjunctivitis symptoms and birch pollen have been performed on a linear basis and have not included air pollution and meteorological variables as potential confounders. The objective of this panel study was to assess the relationship between symptoms of seasonal allergic rhinoconjunctivitis and daily airborne birch pollen concentrations, checking with adequate statistical tools the shapes of concentration-response curves and controlling for confounders. **Methods:** The severity of rhinitis, conjunctivitis, and bronchial symptoms was recorded daily in March and April 2010 (3,311 person-days) in 61 seasonal allergic rhinoconjunctivitis patients sensitized to birch. Data were analyzed with generalized additive and generalized estimating equation models to quantify the effects of birch pollens. **Results:** The relationship between birch pollen and the percentage of patients with nasal, ocular and bronchial symptoms was linear until birch daily average concentrations of, respectively, 110, 70, and 70

grains/m<sup>3</sup>; it reached a plateau thereafter. For an increase of 10 grains/m<sup>3</sup>, the OR (95% CI) for nasal, ocular, and bronchial symptoms were, respectively, 1.07 (1.03–1.12), 1.17 (1.08–1.27), and 1.12 (1.03–1.21). At the beginning of the season, no nasal or ocular symptoms were observed below a threshold of 30 grains/m<sup>3</sup>. **Conclusions:** Adequate modeling regression shows that the clinical response to natural exposure to birch pollen in sensitized patients varies during *Betula* pollen season, with a threshold at the beginning of the season, while over the whole season the relationship is linear for nasal, ocular, and bronchial symptoms up to a saturation point, followed by a plateau.

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## Introduction

Seasonal allergic rhinoconjunctivitis (SAR) is a common disorder with disturbing symptoms affecting the daily quality of life. In large epidemiologic studies, its prevalence was found to be 13% among 10-year-old children in the French Six Cities study [1] and around 20% among adults [2].

Birch pollen is a major source of allergens [3]. Birch trees grow in many natural plant communities, as well as

in urban areas, as ornamentals. In the Scandinavian countries, peak days surpass 3,000 pollen grains/m<sup>3</sup>; in the western and eastern parts of France, they vary between 250 and 2,500 grains/m<sup>3</sup>. The concentrations of airborne pollen are strongly influenced by meteorological conditions, such as temperature, humidity, and sunshine [4].

In patient-based studies, the prevalence of positive skin prick test results to birch in pollen-allergic patients in Europe has ranged from 5% in The Netherlands to 57% in Denmark [5, 6]. In epidemiological studies, the prevalence of birch sensitization in French adults, was 4.7% in the European Community Respiratory Health Survey [7], but it was higher in the eastern part (6.4%) and lower in the southern part (2.3%) of France and 3.5% in 10-year-old French children [1].

Atmospheric pollen counts as measured by Hirst type pollen traps in France are used for monitoring the intensity and length of the pollen season, although they imperfectly reflect the real individual exposure. Using statistical correlation analyses, it has been shown that clinical allergic symptoms are correlated with birch pollen levels [8]. However, modern statistical time series are better fitted to assess the relationship between symptoms and pollen concentrations. Two types of time series studies have been used to assess the epidemiological evidence of the relationship between pollen exposure and SAR or asthma. Ecological time series studies use daily indicators of asthma exacerbations or consultation for rhinitis or conjunctivitis in a population, whereas panel studies relate measurements of pollen grain concentrations to symptom severity in a group of subjects monitored over time.

Some ecological studies have investigated the association between the short-term effects of birch pollen exposure and hospital admission for asthma [9], emergency visits for rhinitis or conjunctivitis [10], antiallergic drug consumption [11, 12], or mortality [13]. A few panel studies have examined the relationship between tree pollens and nasal symptoms [14, 15] and asthma [16–18]. Most studies which have evaluated the short-term effects of *Betula* pollen levels have done so on a linear basis. However, the development of rigorous methods which take into account nonlinear relationships between variables for time series analyses in air pollution studies provides a useful approach for examining the contribution of aeroallergens [19, 20]. Although recent panel grass studies have assessed the effect of pollen and respiratory health using these methods [21, 22], no such studies are available in the case of birch pollen.

This study investigates the dose-response relationship between daily birch pollen exposure in patients sensitized

to *Betula* and daily SAR symptoms. The effects of airborne *Betula* levels on daily observations of SAR symptoms are analyzed using a panel study design, with each subject acting as his or her own control. This instrument is well known to study factors influencing asthma severity [23–26]. To our knowledge, this is its first application in patients suffering from *Betula* SAR and it will help gain a better understanding of clinical responses to *Betula* exposure levels, perhaps allowing better targeted treatments.

## Material and Methods

### Study Population and Allergy Testing

The panel study was conducted in 10 towns in France (Nantes, Angers, Tours, Rouen, Paris, Amiens, Dijon, Clermont-Ferrand, Roussillon, Chambery, Chalon-sur-Saône) plus Geneva (Switzerland). Adult patients sensitized to birch, defined as either a positive skin prick test (Stallergen Diagnostic, Antony, France) and/or a positive specific IgE ( $\geq 0.35$  kU/l) were recruited before the birch pollen season by their allergology specialist. Skin prick tests and/or specific IgE measurements using samples of common allergens (birch, hazel, ash, grass, *Dermatophagoides pteronyssinus*, cat, dog, *Alternaria*) were carried out according to international recommendations [27].

The following eligibility criteria for the study were used: (1) occurrence of seasonal rhinitis coinciding with birch pollen exposure (in March and April), in the past 2 years or longer, diagnosed via 'Allergic Rhinitis and Its Impact on Asthma' criteria [28]; (2) age  $\geq 18$  years; (3) sensitization to birch, and (4) living within 30 km of a pollen captor. The following exclusion criteria were applied: (1) perennial rhinitis; (2) current asthma with daily treatment; (3) sensitization to ash; (4) sensitization to cat and/or mites, with perennial symptoms, and (5) specific immunotherapy for more than 2 years.

On inclusion, each physician completed a questionnaire to collect data on pollinosis history, smoking habits, medical history, and medication, providing detailed instructions on how to fill in the diary.

### Symptom Diary

All participating adults were asked to complete the diary from March 22 to May 16, 2010. They were asked to record, each evening, daily SAR symptoms and any medication taken (antihistaminic, local treatment for conjunctivitis, rhinitis, and asthma). The severity of eye (itching and/or tear flow and/or conjunctival redness), nose (sneezing and/or runny nose and/or blocked nose), and bronchial (cough and/or wheezing and/or asthma) symptoms was recorded on a 4-point scale from 0 to 3 (0 = no symptoms, 1 = slight symptoms, 2 = moderate symptoms, 3 = severe symptoms). Diaries had to be returned to the study site every Monday. If diaries were not returned or if the answers were incorrect (incomplete filling, drug names unspecified or unknown), the subjects were contacted by phone. The study was approved by the Comité Consultatif sur le Traitement de l'Information en matière de Recherche de la Santé (CCTIRS) and all participants provided written informed consent.

### Pollen Data

Average daily birch pollen concentrations were drawn from the National Aerobiological Network Survey (RNSA) in each French city studied and from MeteoSwiss (Geneva). Measurements were made using Hirst type pollen traps, located in each study town, around 15 m above ground levels.

### Air Pollution and Meteorological Data

Air pollution data were obtained in each study town from the Bank Data on Air Quality (BDQA) managed by the Agency for the Environment and Energy Management (ADEME) and from MeteoSwiss (Geneva). The average daily concentrations of NO<sub>2</sub> and PM<sub>10</sub> were used in the study, as well as daily maximum 8-hour average O<sub>3</sub> concentrations. Meteorological data were measured by national meteorology services in each study town.

### Statistical Methods

For each SAR symptom, daily scores were transformed into a dichotomous response variable: 'no symptom' for no symptoms or mild symptoms scored ≤1 (as mild symptoms were frequent even before the start of the birch season), or 'symptoms' for moderate or severe symptoms scored >1.

A two-stage modeling approach was applied with two appropriate models for repeated data, i.e. GAMM and GEE, using a first-order autoregressive correlation matrix. GAMM was used to model the shape of the symptoms-pollen dose-response relationships through the introduction of a spline function. GAMM models were tested using the function gamm (mgcv pack) with a logit link for discrete response in the R software. The GEE model was used to quantify the pollen-symptoms relationships by calculating odds ratios (OR) for an increase of 10 grains of pollens/m<sup>3</sup> with the Genmod procedure in SAS [29]. Where nonlinear relationships between pollens and symptoms were observed, the cut-off level of pollen was determined visually in the GAMM model and a piecewise GEE regression was performed including two quantitative pollen variables: one ranging from 0 to the saturation point and another ranging from the saturation point to the maximum.

Confounding variables, such as pollutants and weather variables, were introduced into the model considering their own effect on both pollen levels and symptoms. They were chosen to avoid highly correlated variables. Maximum temperature, mean relative humidity, wind speed, O<sub>3</sub>, and PM<sub>10</sub> were selected together with age and geographical area of the volunteer (north, west, or east). Two time trend variables were introduced: month ('0' from March 22 to April 16, and '1' from April 17 to May 16) to take into account the medium-term trend in the data, and weekend ('1' vs. '0') to reflect a difference in people's behavior during the weekend (more time spent outdoors). Three different lags were tested: '0' (same day for pollen and symptoms), '1' (relation between day before pollen and symptoms of the day), and '01' (relation between the pollen average of these two days and symptoms of the day), and the result with the most significant lag was retained. Weather and pollution variables were introduced into the model with the same lag as pollen.

Interactions between all explanatory variables were tested and included in the model if significant ( $p < 0.05$ ). Looking for a trigger threshold of symptoms, analysis was restricted to the days of the beginning of the season with pollen below 70 grains/m<sup>3</sup>.

**Table 1.** Characteristics of the population sensitized to *Betula* (n = 61)

|                                                                      | n  | %    |
|----------------------------------------------------------------------|----|------|
| Gender                                                               |    |      |
| Male                                                                 | 31 | 50.8 |
| Female                                                               | 30 | 49.2 |
| Active smoking for at least 1 year                                   |    |      |
| Yes                                                                  | 21 | 34.4 |
| No                                                                   | 40 | 65.6 |
| Passive smoking                                                      |    |      |
| Yes                                                                  | 5  | 8.2  |
| No                                                                   | 56 | 91.8 |
| Sensitized to birch                                                  | 61 | 100  |
| Skin prick test only                                                 | 34 | 56   |
| Skin prick test and specific IgE                                     | 26 | 42   |
| Unknown                                                              | 1  | 2    |
| Sensitized to grass                                                  |    |      |
| Yes                                                                  | 34 | 56   |
| No                                                                   | 27 | 44   |
| Currently being desensitized to birch (<2 years)                     | 34 | 56   |
| Symptoms on more than 4 days a week for at least 1 month last season |    |      |
| Nasal                                                                | 54 | 89   |
| Ocular                                                               | 48 | 79   |
| Respiratory                                                          | 26 | 43   |

## Results

### Study Population

**Panel Participation.** For the panel study, 71 adults with SAR were selected. Ten participants were excluded, either because they failed to provide the requested daily health records (n = 8) or because they did not qualify for inclusion (n = 2). Thus, our analyses are based on data from 61 participants. These 61 volunteers filled in the diary provided for 96% of the 55 maximum possible days on average (from March 22 to May 16, which corresponds to the birch pollinating period). Missing symptom score data occurred on 285 person-days (8.6% of the total expected follow-up of 3,311 person-days) because subjects had left the study area all day, leaving 3,026 person-days of observation. As the duration of each birch pollen period varied between towns, the study period was defined as follows: no birch pollen or <5 daily grains/m<sup>3</sup> before the first day and all cities presenting several consecutive days with 0 grains at the end.

**Panel Description.** Table 1 describes the characteristics of the population. The mean age (SD) of the study population was 41.6 years (12.2); their mean age at the beginning of the SAR disease was 23 years (14), and the mean duration of SAR was 18 years (15).

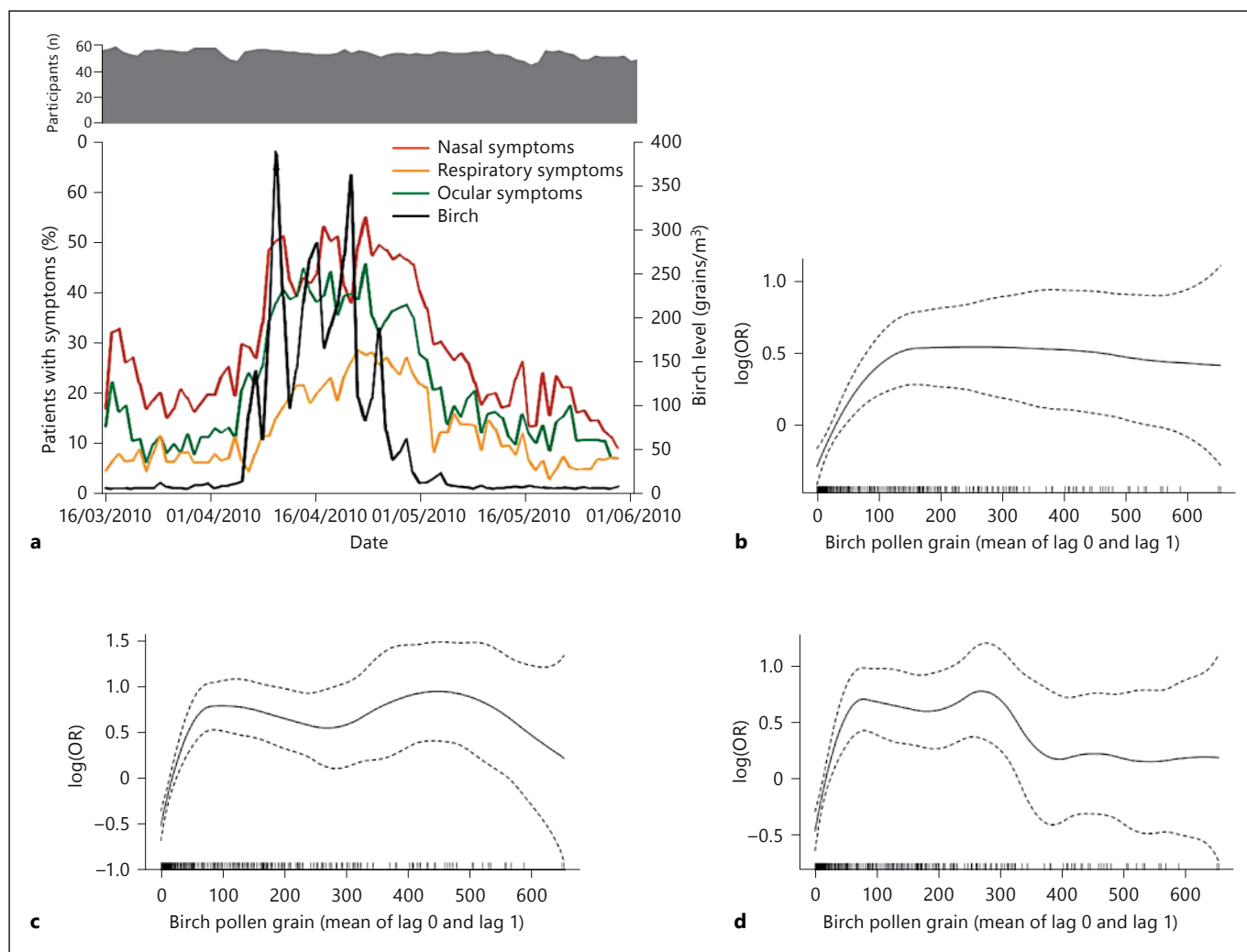


**Fig. 1.** **a** The percentage of patients with moderate and severe symptoms is on the y-axis and pollen level (weighted by the number of patients answering per city per day) vs. time is on the x-axis. The top shows the number of participants on each day. Shape of the pollen-symptoms curve from the GAMM models for, respec-

tively, the nasal (**b**), ocular (**c**), and respiratory symptoms (**d**). The y-axis represents the part of the symptom percentage due to pollen (in logit). The pollen-symptoms relation [expressed as  $\log(\text{OR})$ ] can be read as the symptom increase on the y-axis for a pollen increase on the x-axis.

**Symptom Diary.** Figure 1a shows the daily percentage of patients with nasal, ocular and respiratory moderate or severe symptoms during the observational study period.

#### Exposures

The evolution of daily pollen levels during the birch pollen season is displayed in figure 1a. These levels are weighted according to the number of participants per town and per day. The sudden release of *Betula* at the beginning of April, clearly shown in fig. 1a, is present in all towns.

#### Pollen-Symptoms Regression Models

Figure 1b–d displays the shape (with 95% CI) of the pollen-symptoms curves from the GAMM models, adjusted for correlated response, time trend, pollutants, and meteorological variables. The effect of a pollen unit increase on the x-axis leads to a symptom increase on the y-axis expressed as ‘ $\ln(\text{OR})$ ’ (natural logarithm of the OR). The plot suggests that the effect of *Betula* pollen on nasal, ocular, and bronchial symptoms was more or less linear, up to a saturation point of 110, 70, and 70 grains/m³, respectively. Above these cut-offs, the effects of pollens on these symptoms remain

**Table 2.** OR (95% CI) of the effect of a 10 grains/m<sup>3</sup> increase in birch pollen on moderate and severe symptoms during the whole season (GEE model)

|                                                         | Birch ≤cutoff    | Birch >cutoff    |
|---------------------------------------------------------|------------------|------------------|
| Nasal symptoms (cutoff = 110 grains/m <sup>3</sup> )    | 1.07 (1.03–1.12) | 1.00 (0.99–1.01) |
| Ocular symptoms (cutoff = 70 grains/m <sup>3</sup> )    | 1.17 (1.08–1.27) | 1.00 (0.99–1.00) |
| Bronchial symptoms (cutoff = 70 grains/m <sup>3</sup> ) | 1.12 (1.03–1.21) | 0.99 (0.98–1.00) |

**Table 3.** OR of the effect of a 10 grains/m<sup>3</sup> increase below the cutoff in birch pollen on moderate and severe symptoms during the whole season (GEE model)

|                 | Person-days | Nasal symptoms<br>(pollen ≤110 grains/m <sup>3</sup> ) |           | Ocular symptoms<br>(pollen ≤70 grains/m <sup>3</sup> ) |           | Respiratory symptoms<br>(pollen ≤70 grains/m <sup>3</sup> ) |           |
|-----------------|-------------|--------------------------------------------------------|-----------|--------------------------------------------------------|-----------|-------------------------------------------------------------|-----------|
|                 |             | OR                                                     | 95% CI    | OR                                                     | 95% CI    | OR                                                          | 95% CI    |
| Complete model  | 3,311       | <b>1.07</b>                                            | 1.03–1.12 | <b>1.17</b>                                            | 1.08–1.27 | <b>1.12</b>                                                 | 1.03–1.21 |
| Desensitization |             |                                                        |           |                                                        |           |                                                             |           |
| Yes             | 1,827       | 1.04                                                   | 0.99–1.09 | <b>1.14</b>                                            | 1.05–1.25 | <b>1.15</b>                                                 | 1.02–1.30 |
| No              | 1,484       | <b>1.12</b>                                            | 1.05–1.20 | <b>1.20</b>                                            | 1.06–1.36 | 1.08                                                        | 0.95–1.22 |

Numbers in bold denotes significance.

relatively constant but with low accuracy due to limited data.

Piecewise GEE models quantified, for an increase of 10 grains/m<sup>3</sup>, significant pollen-symptom relationships up to the saturation points for nasal, ocular, and bronchial symptoms, respectively [OR = 1.07 (95% CI 1.03–1.12), OR = 1.17 (95% CI 1.08–1.27), and OR = 1.12 (95% CI 1.03–1.21)]. Beyond these cutoffs, OR are lower and not significant (table 2). In table 3, results are given after stratification according to specific immunotherapy, with increased risks for nasal and ocular symptoms in subjects without immunotherapy compared to subjects with recent immunotherapy, while the opposite is true for respiratory symptoms.

#### *Beginning of the Season (0 < Birch Pollen < 70 grains/m<sup>3</sup>)*

At the start of the season (i.e. before pollen levels reached 70 grains/m<sup>3</sup>), the GAMM model (fig. 2) shows a threshold of 30 grains/m<sup>3</sup> (i.e. 20 grains/m<sup>3</sup> for lag 01) for the relationships between pollens and nasal and/or ocular symptoms.

## Discussion

Complex relationships exist between pollen exposure and symptoms [8]. In our panel study, the dose-response curve for nasal, ocular, and bronchial symptoms estimated

globally during the pollen season was linear up to a pollen value of 110, 70, and 70 grains/m<sup>3</sup>, respectively, followed by a plateau. At the beginning of the season, no symptoms were observed below a threshold of 30 grains/m<sup>3</sup>.

Studies on dose-response relationships between pollen concentrations and clinical symptoms are scarce. Some studies have demonstrated varying degrees of correlation between symptoms and birch counts [15]. Different threshold values, in subjects with hay fever, have been mentioned for the onset of symptoms. In a panel study conducted in Turku, Finland, in 1978, Viander and Koivikko [16] suggested a threshold of 30 birch grains/m<sup>3</sup> based on correlational statistical analyses. Our study, using more adequate time statistical tools, confirms this threshold of 30 grains/m<sup>3</sup>. In the present study, we did not consider the problem of the variability of clinical sensitivity and we tried to define a pollen threshold value useful for predicting the onset of clinical symptoms in most allergic patients.

In early studies, it was observed [16] that the number of birch pollen grains necessary to induce respiratory symptoms in about 90% of sensitized patients was 80 grains/m<sup>3</sup>. Ten years later, Taudorf and Moseholm [17], using time series analysis, showed that the association between birch pollen load and symptom severity was non-linear, with a plateau. Our study distinguishes a nasal plateau of 110 grains/m<sup>3</sup> and an ocular and bronchial plateau of 70 grains/m<sup>3</sup>.

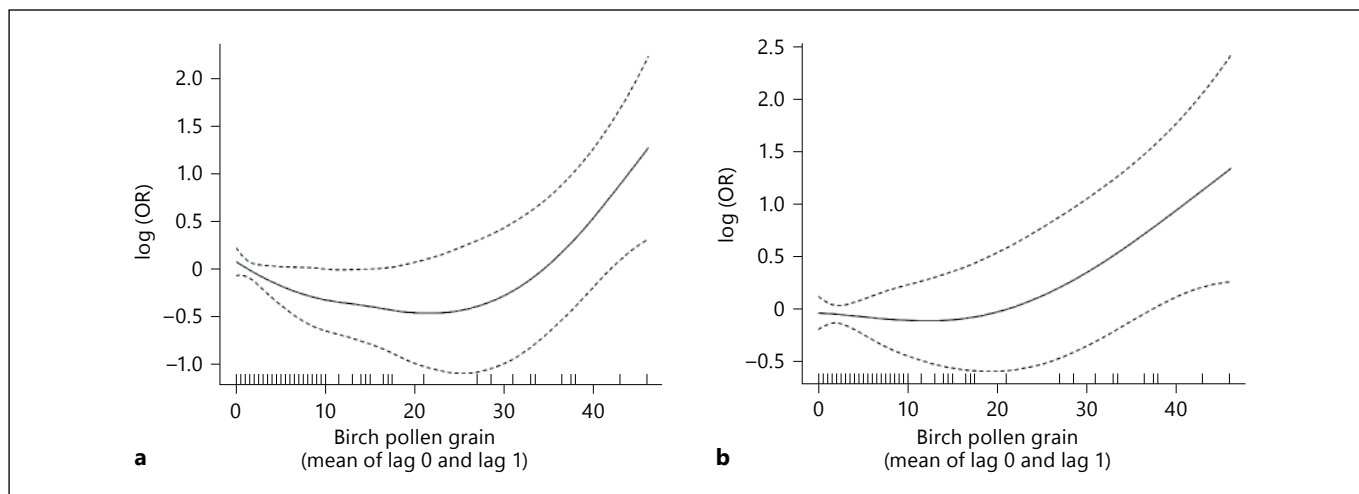


**Fig. 2.** Shape of the pollen-symptoms curve from the GAMM models for, respectively, the nasal (**a**) and ocular symptoms (**b**) at the beginning of the season when pollens remain below 70 grains/m<sup>3</sup> (lag 01 of pollen <50 grains/m<sup>3</sup>). The y-axis represents

the part of the symptoms percentage due to pollen (in logit). The pollen-symptoms relation [expressed as log(OR)] can be read as the symptom increase on the y-axis for a pollen increase on the x-axis.

The lag 01 observed for birch effect in this study is in line with those described in previous studies (Viander and Koivikko [16] and Huynh et al. [30], lag 0, and Taudorf and Moseholm [17], lag 1), even if a few authors found longer lags (lag 2 [31] and lag 3 [32]).

We did include medication use as an explanatory variable in our models but, as it did not change the results, medications were not retained (data not shown). In addition, medication records are not necessarily more objective than the report of symptoms, since a number of factors unrelated to symptom severity also influence use.

One of the strengths of this study is its statistical power, as repeated measurements, combined with the fact that each subject acted as his or her own control, meant that our sample of 61 adults contributed to 3,026 person-days of observation. Some limitations of this study include that pollen exposure was based on data from a single pollen sampler in each town. A single collector can nevertheless represent the temporal variation of pollen concentration in a given species within a 30-km radius [33] and, although rooftop pollen counts do not completely reproduce human exposure at street level, personal exposure to pollen correlates well with stationary measurements used to report pollen counts [34]. Stationary measurements were used for assessing air pollution; studies on exposure of air pollution indeed show that in longitudinal analysis the correlation between personal exposure and stationary measurements is better than in cross-

sectional analysis [35]. Another limitation of this study is that a skin prick test and/or specific IgE was used to assess sensitization. Skin prick tests have, however, been proven to produce results comparable to specific IgE for detecting IgE-mediated allergy diseases [36]. No information was collected on fungal spores, although they have been associated with respiratory symptoms. However, usually, fungal spores are found at high levels in the atmosphere only after Betulaceae season. Moreover, in French epidemiological studies, sensitization to molds has been rather low in adults [7] and in children [37]. Our patients might not represent the general population suffering from birch hay fever but rather the type of patients with severe symptoms typically seen by allergists. Finally, airborne birch pollen counts may not be representative for allergen exposure and may be a single proxy of exposure. In a recent European study of release of the major birch pollen allergen Bet v 1 [38], a >10-fold difference in daily allergen release per pollen was measured. However, in a Spanish study [39], these two biological parameters (*Olea europea* pollen counts and allergen exposure) were significantly correlated with the symptom scores of allergic patients. Future studies monitoring the Bet v 1 allergen itself together with birch pollen counts would be of interest in assessing the accurate relationship between clinical symptoms and birch exposure.

In conclusion, the clinical response in sensitized patients to natural exposure to birch varies. Our data suggest a threshold at the beginning of the season. Over the

whole season, the relation is firstly linear for nasal, ocular, and bronchial symptoms up to a saturation point, followed by a plateau.

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