POSITION PAPER

Defining pollen exposure times for clinical trials of allergen immunotherapy for pollen-induced rhinoconjunctivitis – an EAACI position paper

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Abstract

Background: Clinical efficacy of pollen allergen immunotherapy (AIT) has been broadly documented in randomized controlled trials. The underlying clinical endpoints are analysed in seasonal time periods predefined based on the background pollen concentration. However, any validated or generally accepted definition from academia or regulatory authorities for this relevant pollen exposure intensity or period of time (season) is currently not available. Therefore, this Task Force initiative of the European Academy of Allergy and Clinical Immunology (EAACI) aimed to propose definitions based on expert consensus.
Methods: A Task Force of the Immunotherapy and Aerobiology and Pollution Interest Groups of the EAACI reviewed the literature on pollen exposure in the context of defining relevant time intervals for evaluation of efficacy in AIT trials. Underlying principles in measuring pollen exposure and associated methodological problems and limitations were considered to achieve a consensus.
Results: The Task Force achieved a comprehensive position in defining pollen exposure times for different pollen types. Definitions are presented for 'pollen season', 'high pollen season' (or 'peak pollen period') and 'high pollen days'.
Conclusion: This EAACI position paper provides definitions of pollen exposures for different pollen types for use in AIT trials. Their validity as standards remains to be tested in future studies.

Evidence for the efficacy of pollen allergen immunotherapy (AIT) is based on randomized controlled trials (RCTs) using clinical endpoints such as the combined symptom and medication score (CSMS) as proposed by the EAACI Immunotherapy Interest Group-Task Force (1, 2), the US Food and Drug Administration (FDA) (3) and the European

Table 1 Examples of heterogeneous definitions for grass pollen season and peak-grass pollen season in three different allergen immunotherapy trials

Season	Peak season	Reference
'First day of 3 consecutive days with a grass pollen count above 30 grass pollen grains/m^3 of air to the last day before 3 consecutive days with a pollen count below 30 grains/m^3'	No information	Didier et al. JACI 2007 (63)
'First day of 3 consecutive days with a pollen count equal to or above 10, to the last day before 3 consecutive days with a pollen count less than 10'	'A period of 15 days with the highest moving average'	Durham et al. JACI 2006 (64)
'The start of the pollen season was defined as when the grass pollen count was ≥5 pollen grains/m^3 per day for more than 3 consecutive days. Efficacy was evaluated until the pollen count fell to ≤5 pollen grains/m^3 per day for 3 consecutive days, indicating the end of the relevant pollen exposure'	'…1 week before and 2 weeks following the day of maximum grass pollen exposure in the respective season'	Pfaar et al. Allergy 2012 (65)

Medicines Agency (EMA) (4). In RCTs, these endpoints are analysed in relation to predefined time points that encompass either the entire pollen season or a predefined peak pollen season.

Airborne pollen is sampled using devices such as Hirst-type volumetric spore traps (5–7) and expressed as pollen concentrations as the number of pollen per cubic meter of air. The relevant local pollen exposure is usually presented as daily mean concentrations that may be used to define the relevant time window for evaluation of therapeutic interventions such as AIT for seasonal allergic rhinoconjunctivitis (ARC).

In the current 'Guideline on the Clinical Development of Products for Specific Immunotherapy for the Treatment of Allergic Diseases' (4), the EMA recommends that in RCTs there should be a clear definition 'in the study protocol (of) the minimum pollen count which has to be reached to define the evaluation period as well as the baseline period'. However, any validated or generally accepted definition for this relevant pollen exposure intensity or period of time (season) is still lacking as well as strict consensus/direction from academia or regulatory authorities. As a consequence, there is a number of different methodologies for defining a 'pollen season', the start or end of a season or the definition of a 'peak pollen season' (8–11) (examples in Table 1).

Likewise, there is also no clear definition for the level of 'relevant' indoor allergen exposure such as house dust mites or animal dander for analysis of treatment efficacy in RCTs. A World Allergy Organization (WAO) Taskforce on 'Recommendations for standardization of clinical trials with Allergen Specific Immunotherapy for respiratory allergy' emphasized that for these allergens there should be a report of the relevant exposure in AIT trials (baseline, during and end of trial) although no precise definitions were suggested (9).

A clinically relevant and scientifically justified/evaluated threshold of pollen allergen exposure (e.g. minimal amount of airborne pollen that is necessary to trigger a nasal or conjunctival allergic reaction) has not yet been defined throughout the literature (12). Moreover, such a definition must be a compromise as it is known that the threshold of pollen exposure for symptoms in allergic individuals may vary widely (8). A first review of attempts to correlate exposure to symptoms was conducted under the *European Cooperation in the field of Scientific and Technical Research (COST)* action ES0603 (13, 14). From this report, it was not possible to draw conclusions, and clearly more research is necessary. In the absence of standardized definitions direct comparisons between different RCTs on the efficacy of AIT with pollen extracts and meta-analysis of such trials must be suboptimal due to inevitable heterogeneity. In addition, allergen exposure is reported as pollen/m^3, and recent literature suggests a likely discrepancy between pollen/m^3 and the actual magnitude of release of allergenic proteins from the pollen (15–17).

The EAACI Immunotherapy and the EAACI Aerobiology and Pollution Interest Groups have conducted a Task Force (TF) on '*Definition of clinical-relevant thresholds of allergen-exposure for analysis of outcomes in AIT*'. The aim was to define and recommend the periods that are crucial for pollen exposure and pollen-induced symptoms. Based on the proposed definitions, future protocols of randomized controlled clinical trials on AIT for ARC may be better harmonized and, therefore, comparable.

Abbreviations

AIT, allergen immunotherapy; ARC, allergic rhinoconjunctivitis; COST, European Cooperation in the field of Scientific and Technical Research; CSMS, combined symptom and medication score; EAACI, European Academy of Allergy and Clinical Immunology; EAN, European Aeroallergen Network; EMA, European Medicines Agency; FDA, Food and Drug Administration; HDM, house dust mite; PHD, patient's hay-fever diary; PP, position paper; RCT, randomized controlled (clinical) trial; SIT, specific immunotherapy; SLI, symptom load index; TF, Task Force; WAO, World Allergy Organization.

Methodology

The first workshop on *'thresholds in AIT'* was held on 07 June 2014 during the annual EAACI-congress in Copenhagen, Denmark. The primary objectives were (i) to review current definitions of clinically relevant exposures (thresholds and time periods for evaluation, including regulatory views) used in RCTs of AIT, (ii) to achieve consensus for a standardized definition that captures the relevant time period for patients in different regions and for the different pollen types and (iii) to identify unmet needs for future research.

A second TF meeting was held in Zürich, Switzerland, on 07 November 2014 when subgroups of the TF drafted sections on different definitions of pollen threshold level and relevant time periods for analysing outcomes in AIT trials. During the final TF consensus meeting in Zürich, Switzerland, on 6 May 2015 all sections were discussed and revised and a consensus on definitions was achieved. Thereafter, the committee drafted a Position Paper (PP) that was circulated to all TF members for critical review and final approval.

Results

This PP reviews the literature on pollen exposure used in defining time intervals for evaluation of efficacy in AIT trials. The underlying principles for measuring pollen exposure, and methodological problems and limitations are considered. Finally, recommendations for the definition of relevant pollen exposures are given along with the rationale for these definitions.

The regulatory and academic view

An important EMA guideline on the clinical development of products in AIT highlighted that 'In case of seasonal allergies it is mandatory to document the exposure to the relevant allergens and to define in the study protocol the minimum pollen count which has to be reached to define the evaluation period as well as the baseline period' (4). In a statement of the WAO on 'the standardization of clinical trials with Allergen Specific Immunotherapy for respiratory allergy' (9), it is defined that: 'The time of exposure to allergens is highly variable and depends on the allergen. For pollen allergy, the pollen count is important and the clinical effects of SIT (specific immunotherapy, *the authors*) should be recorded during the entire pollen season. However, the primary outcome analysis can be made for clearly identified relevant periods, e.g. for weeks when the pollen load is above a predetermined level'. With regard to allergen exposure, this PP states: 'Allergen exposure should be monitored during any SIT trial. Pollen counts using the same method and distributed evenly according to the patients' distribution should be studied. Aerobiological data from the nearest independent monitoring station will usually be sufficient' (9).

These definitions are not precise and therefore not useful for direct guidance during studies on immunotherapy. The COST action ES0603, for the first time, established a multidisciplinary forum that critically reviewed the existing information on pollen production, release and transport in Europe (13, 18). The development and widespread clinical use of electronic personalized hay fever diaries (19) in AIT trials have allowed the opportunity to combine such information with pollen data in a wide variety of patients and to evaluate the importance of this information.

For example, a combination of monitoring and fine-scale modelling of pollen sources, emission and dispersal, with an appropriate dispersion model, could improve the evaluation of exposures at different locations.

General considerations

Different definitions

The proposed definitions in this PP, for example of the pollen season, are focused to define the time period in which placebo- and active-treated groups in AIT studies should be compared. Consequently, they may not be appropriate for other purposes. For instance, definitions have been used to study the influence of meteorological and climatological factors on pollen concentrations (20–24) or to broadcast information to the public. In this latter setting, pollen data serve several aims. First, to inform the public about the presence of pollen in the air, but more importantly to give an accurate prediction of the risk for symptom development (in pollen-allergic patients) in terms of classes of risk (no risk, moderate risk and high risk) (25). Studies in this area focus on development of models based on forecasted temperature and grass pollen/m^3 to predict hay fever symptoms up to 5 days ahead (26, 27) or more complex numerical models to take into account atmospheric processes and the liberation, dispersal and deposition of pollen (28–30).

Principles in measuring daily pollen concentrations

Mean daily pollen concentrations for a specific pollen type record the number of pollen per cubic meter of air averaged over a 24-h sampling period (5, 31). In this PP, pollen concentrations are expressed as pollen/m^3. In the literature, the term '*daily pollen count*' is frequently used to express the daily mean pollen concentration. This term originates from the fact that when analysing measurements of Hirst-type pollen traps, pollen are counted on microscope slides. These raw counts are dependent on the method of counting and have no meaning by themselves. For getting comparable pollen data, the number of counted pollen is then converted into pollen concentrations. In conclusion, we draw attention to the fact that the term 'daily pollen count' is not correct and recommend to use in future 'daily mean pollen concentration' or in a short form 'daily pollen concentration'.

The current standard sampling instrument in all European pollen networks is a Hirst-type volumetric pollen trap (32). The trap contains a motor, a vacuum pump, a rotating drum with coated tape, a clockwork motor and a wind vane. These pollen traps sample air at a fixed rate by aspiration (flow rate 10 l/min). This allows the continuous impaction of pollen/fungal spores/particles on the sticky surface of the coated tape. The sampler is placed on a readily accessible, flat, horizontal surface, often the roof of a building. The trap should

not be placed near fixed or mobile sources of mass emission of biological or nonbiological particles. After exposure, the coated tape is collected and, in the laboratory, cut and placed on glass slides. Light microscopy is used for identification and counting of the pollen. It allows evaluating pollen concentration data at a temporal resolution of 2 h and more. Minimum requirements for pollen sampling, use of material and reagents and analysing the microscope slides for European pollen networks are given by Galán et al. (33). The sampling and analysis methods based on Hirst's method (32) are in the final process of standardization for all European countries (34) and described already as guideline for Germany in detail (35).

Limitations of pollen data

Few studies have evaluated whether pollen concentrations from one pollen sampler may be representative of exposures at distant locations; pollen sources are rarely mapped in fine scale. However, some studies have shown that similar patterns of pollen exposure can be found over large biogeographical areas (36, 37), if climate/weather, vegetation and altitude are similar (33, 38). Empirically, most Hirst-type pollen traps are positioned at roof top level and are assumed to be representative for a given area [e.g. a radius of 30 km (39)]. A few studies have investigated the correlation between pollen concentrations at rooftop level (10–15 m) compared to street level (1.5 m). The pollen concentrations were well-correlated, but generally higher at ground compared to rooftop level, especially for grasses and herbs. The presence of local plants can influence the ground-level pollen concentrations (40–42). *Artemisia* and grass pollen were detected 1–2 weeks earlier at ground level than on the roof (42). In street canyons, an important spatial variability was found, but the difference in grass pollen concentration was small and was found to depend on weather conditions (43) leading to higher grass pollen concentrations at higher humidity at street level (39, 44). Crucially, the results of one pollen sampler of reference for a given area will not capture the variable exposures among patients because of their different activities, different timings and durations of outdoor activity, different locations, etc. (45, 46). Clearly for accurate determination of personal exposures, the development and use of a standardized personal pollen sampler would be an advantage (47).

Finally, one has to bear in mind that the average measurement error with a Hirst-type trap amounts to 25% (variability increasing with lower pollen concentrations) (16, 33, 48). Thresholds of allergen exposure triggering an allergic reaction are typically in a relatively low range of the measured concentrations (between 0 and 100 pollen/m^3). Further information can be found in several publications, for example (49, 50).

Relationship between allergic symptoms and pollen concentrations

Pollen-allergic patients have symptoms during the pollen season, and this temporal association is critical for accurate diagnosis (5, 14).

On a patient group basis a good correlation is observed between the level of daily symptoms and the pollen concentrations (49–52). However, it is known that grass pollen-allergic patients have more severe symptoms in the early pollen season compared to the late season at similar grass pollen concentrations, likely due to a 'priming effect' due to early exposures lowering the pollen threshold for the occurrence of subsequent symptoms (51, 52).

New internet-based tools for collecting patient's symptoms data have been available only in recent years (e.g. www.pollendiary.com, www.allergieradar.nl). The opportunities to study high numbers of participants provided already valuable information about the impact of pollen concentrations on the kind and severity of symptoms (52–54). The influence of other factors, for example weather factors (wind, rain, etc.) or the duration of time users spent indoors or outdoors, will be proven in the future. This is the first step towards the pollen forecast of the future, aimed at proposing an individual symptom forecast for pollen allergy sufferers.

A *symptom load index (SLI)* was developed based on the data pool of the patient's hay-fever diary (PHD) (11). The pollen season severity described by the SLI changes when comparing different pollen seasons or countries and is not always correlated with the pollen concentrations. Thus, the same amount of pollen can induce different symptom levels in different years and in different regions (11).

Airborne allergens vs *pollen concentrations*

Due to the currently available pollen monitoring over the whole of Europe (55) and the lack of sufficient data on the actual concentrations of airborne allergens, definitions must depend on daily pollen concentrations and not allergen concentrations. Under specific meteorological conditions, for example, high humidity, allergens can bind to particles smaller than pollen and under certain conditions be released from pollen (16, 56). In addition, the question of the variability of airborne concentrations of the major allergens, for example Bet v 1 (57), Ole e 1 (17) or Phl p 5 (16), is not yet fully understood and there is no data concerning concentrations of the minor allergens of birch, olive or grass pollen. Therefore, standardized assays and more field studies are needed to address these questions.

In view of these limitations, the only currently available way of relating symptoms and rescue medication with exposure to pollen allergens remains a comparison with airborne pollen/m^3.

Why is it necessary to include in the definition days with very low concentrations at the beginning of the pollen season?

Patient's hay-fever diary data indicate that pollen allergy symptoms are present very early in the season, even on days with very low pollen concentrations (Figures 1 and 2). Although speculative, this effect might be attributable to the following:

1 Distance of traps from pollen sources (representativeness of the pollen trap) and exposure to local pollen sources. As discussed above, the position of a pollen trap is crucial for its potential to represent a certain area or region. Local pollen production will vary, but this important factor is averaged during atmospheric transport to the monitor. Pollen could indeed be released very locally due to

particular plant genotypes or environmental conditions that provide added burden to pollen allergy sufferers not represented by regional pollen/m^3 recorded remotely. Such effects are known and documented, for example, for the flowering of *Alnus* × *spaethii* around Christmas in Switzerland (58) and Austria (59).

2 Exposure to other pollen carrying the same allergen (e.g. pollen of the same botanical family such as *Carpinus* sp. and *Betula* sp.).
3 Psychological reasons linked to pollen forecasts that announce the start of the season – such information might trigger enhanced awareness of pollen allergy and – possibly – exaggerate anticipation of symptoms.
4 Other reasons: The influence of the allergen-free interval before the season. Whereas patients with perennial allergy may have blunted awareness with constant exposure to perennial allergens, for hay fever sufferers the first exposure to a seasonal allergen following a prolonged period of time without allergen contact may trigger more symptoms at low allergen concentration compared to no allergen for the rest of the year. Alternatively, early symptoms could be triggered by the presence of other allergens in the air (allergy to different allergens). In northern Europe where patients are frequently dual sensitized to both birch and grass pollen, it is likely that the priming effect of birch pollen increasing local allergic inflammation may lower the threshold for grass pollen-induced symptoms.

These observations led the TF to explicitly exclude definitions based on a percentage of the annual sum of daily pollen concentrations, because they result in variable exposure levels

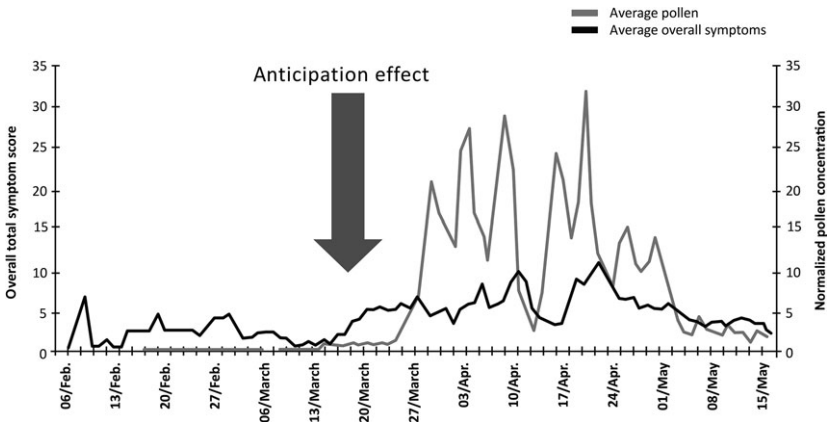

Figure 1 *Betula* pollen and reported symptoms on nose, eye and bronchi in 2010 in Austria. Possible anticipation effect highlighted: allergy sufferers may 'expect' symptoms at the beginning of the announced season. The overall total symptom score is based on the 'symptom load index (SLI)' (11), detailed description in section 'Relationship between allergic symptoms and pollen concentrations'.

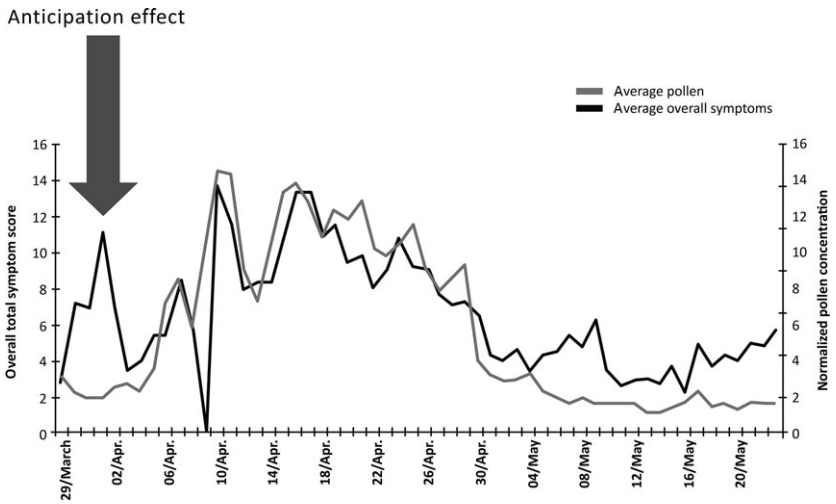

Figure 2 *Betula* pollen and reported symptoms on nose, eye and bronchi in 2010 in France. Possible anticipation effect highlighted: allergy sufferers may 'expect' symptoms at the beginning of the announced season. The overall total symptom score is based on the 'symptom load index (SLI)' (11), detailed description in section 'Relationship between allergic symptoms and pollen concentrations'.

at the beginning and the end of the season with regard to the concentrations of pollen over the whole season. Even years with very low pollen concentrations, for example, 40 grass pollen day/m^3 = 100% of the seasonal pollen load would have a start and an end of a 'season' which would be used in AIT.

For reasons mentioned in (2) and (4), it is therefore recommended to analyse also the other pollen types present in the atmosphere before and during the RCTs.

Alternative or complementary method: the use of the comparator group in trials for the definition of a 'relevant' season ('placebo-based approach')

In AIT studies with pollen extracts, allergen exposure is typically assessed by pollen concentration data during the whole season or in peak pollen periods, but these may misrepresent exposure if performed remotely from multiple study centres.

It was assessed whether symptomatology in placebo-treated patients is a better measure of local allergen burden at individual centres than the pollen concentrations in such trials (60, 61). Data from a multicentre, placebo-controlled trial of preseasonal grass pollen AIT (62) were reanalysed (*post hoc* analysis) to identify the 4 weeks at each centre in which the placebo-treated subjects had the highest CSMS (60). The difference in CSMS between active and placebo groups was compared during the 4 peak placebo score weeks (analysis 1) and during the 4 peak pollen concentration weeks (analysis 2). The effect size of AIT over placebo was higher in analysis 1 (18.5%) compared to analysis 2 (13.6%), with increased statistical significance ($P = 0.0001$, $P = 0.0038$, respectively). Similar improved discrimination was observed when analysing benefits in subgroups of patients with severe symptoms, a high disease burden and in different geographical locations.

If this novel analysis creates better discrimination of the effects of AIT compared with placebo, it may be suitable as an additional secondary/exploratory endpoint that takes into account some of the limits of pollen data: *that is,* reproducibility, distance to trap, dead zones, different trap performances, and differences between pollen concentrations and major allergen release (16). However, it should be clearly emphasized that at present such a *post hoc analysis* is not accepted by regulatory bodies such as the EMA and this approach cannot replace the whole season and peak season based on the analysis of pollen/m^3 (4).

Proposed definitions

Consented definitions for the periods of pollen exposure times for analysis of outcomes in clinical trials of AIT in ARC due to pollen are given in Table 2. The selection of the amounts of different pollen was based on existing threshold values for symptom development (14), the comparison between pollen data from the European Aeroallergen Network (EAN) database (https://ean.polleninfo.eu/) and 'real-life' data as reported by patients using the online patient's hay-fever diary (https://www.pollendiary.com/) and the Pollen App 4.1 (https://www.pollenwarndienst.at/de/gratis-pollen-app.html) to document their actual (daily) symptoms on eyes, nose and bronchi during pollen exposure within the season (19, 52). As such, these definitions have been elaborated by the TF experts based on data belonging to central and southern Europe, well knowing that values used in the definitions might need adaptation to different biogeographical regions. The validation of these definitions should be prospectively performed with symptoms data from patients. However, this is the first international position from a panel of experts to our best knowledge.

Conclusion

Allergen immunotherapy trials for seasonal pollinosis depend on an accurate knowledge of (pollen) exposure times. Exposure is estimated by the measure of airborne pollen concentrations (number of pollen in m^3 of air) by standardized monitoring stations. Although these data are fundamental, it is a methodological challenge that will take years to establish a definition that captures the relevant time period for patients in different regions and for different pollen types. Possible solutions include a more detailed correlation analysis of symptoms, medications, pollen concentrations and measures of free allergens (released from pollen). Definitions of seasonal exposures will need adaptations depending on the setting for which they are required.

The Task Force of the Immunotherapy and Aerobiology and Pollution Interest Groups of the European Academy of Allergy and Clinical Immunology (EAACI) highlights consented recommendations on the definitions for different kinds of pollen in future AIT field trials.

The definitions given from our TF are largely empirical, based on expert consensus (i.e. category D evidence) as supported from (retrospective) data from EAN database and real-life data on symptom severity, and should be regarded as tentative and for critical evaluation. Our proposed definitions may be analysed for their sensitivity in two ways: (i) how does modifying definitions of pollen exposure affect the correlation between symptoms and pollen concentrations and (ii) how does modifying definitions of pollen exposure affect the ability to detect a treatment effect between groups in double-blind, placebo-controlled studies on AIT.

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Conflicts of interest

Dr. Pfaar reports grants and personal fees from ALK Abelló, grants and personal fees from Allergopharma, grants and personal fees from Stallergenes, grants, personal fees and nonfinancial support from HAL-Allergy, grants and personal fees from Bencard Allergie, grants and personal fees from Lofarma, grants from Biomay, grants from Nuvo, grants from Circassia, grants and personal fees from Biotech Tools S.A., grants and personal fees from LETI Pharma, grants and personal fees from Novartis, personal

Table 2 Overview on proposed definitions of periods of pollen exposure times for analysis of outcomes in allergen immunotherapy in allergic rhinoconjunctivitis due to pollen.* Daily mean pollen concentration (pollen/m^3) is used for these definitions

	Pollen season	High pollen season (or 'Peak pollen period')†	High pollen days
Birch (*Betula* sp.)	Start of season: 1st day of 5 days (out of 7 consecutive days) each of these 5 days with ≥10 pollen/m^3 and with a sum of these 5 days of ≥100 pollen/m^3 End of the season: last day of series of 5 days (out of 7 consecutive days) with ≥10 pollen/m^3 and with a sum of these 5 days of ≥100 pollen/m^3	Start of the peak pollen period: 1st day of 3 consecutive days, each with at least ≥100 pollen/m^3 End of the peak pollen period: last day of at least 3 consecutive days, each with ≥100 pollen/m^3	The day(s) with at least 100 pollen/m^3
Grasses (Poaceae)	Start of season: 1st day of 5 days (out of 7 consecutive days) each of these 5 days with ≥3 pollen/m^3 and with a sum of these 5 days of ≥30 pollen/m^3 End of the season: last day of series of 5 days (out of 7 consecutive days) with ≥3 pollen/m^3 and with a sum of these 5 days of ≥30 pollen/m^3	Start of the peak pollen period: 1st day of 3 consecutive days, each with at least ≥50 pollen/m^3 End of the peak pollen period: last day of at least 3 consecutive days, each with ≥50 pollen/m^3	The day(s) with at least 50 pollen/m^3
Cypress (*Cupressus* sp.‡)	Start of season is defined as 1st day of 5 days (out of 7 consecutive days) each of these 5 days with ≥20 pollen/m^3 and with a sum of these 5 days of ≥200 pollen/m^3 End of the season: last day of series of 5 days (out of 7 consecutive days) with ≥20 pollen/m^3 and with a sum of these 5 days of ≥200 pollen/m^3	Start of the peak pollen period: 1st day of 3 consecutive days, each with at least ≥100 pollen/m^3 End of the peak pollen period: last day of at least 3 consecutive days, each with ≥100 pollen/m^3	The day(s) with at least 100 pollen/m^3
Olive (*Olea* sp.)	Start of season is defined as 1st day of 5 days (out of 7 consecutive days) each of these 5 days with ≥20 pollen/m^3 and with a sum of these 5 days of ≥200 pollen/m^3 End of the season: last day of series of 5 days (out of 7 consecutive days) with ≥20 pollen/m^3 and with a sum of these 5 days of ≥200 pollen/m^3	Start of the peak pollen period: 1st day of 3 consecutive days, each with at least ≥100 pollen/m^3 End of the peak pollen period: last day of at least 3 consecutive days, each with ≥100 pollen/m^3	The day(s) with at least 100 pollen/m^3
Ragweed (*Ambrosia* sp.)	Start of season is defined as 1st day of 5 days (out of 7 consecutive days) each of these 5 days with ≥3 pollen/m^3 and with a sum of these 5 days of ≥30 pollen/m^3 End of the season: last day of series of 5 days (out of 7 consecutive days) with ≥3 pollen/m^3 and with a sum of these 5 days of ≥30 pollen/m^3	Start of the peak pollen period: 1st day of 3 consecutive days, each with at least ≥50 pollen pollen/m^3 End of the peak pollen period: last day of at least 3 consecutive days, each with ≥50 pollen pollen/m^3	The day(s) with at least 50 pollen/m^3

*These definitions are based on comparisons between data from European Aeroallergen Network database and real-life data (19, 52) and knowledge about threshold levels for symptom development (14).
†Multiple peak pollen periods may occur during one pollen season.
‡The definition for this pollen is only valid for Mediterranean areas where *Cupressus* species dominate the Cupressaceae concentrations.

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