



Contents lists available at ScienceDirect

World Allergy Organization Journal

journal homepage: www.sciencedirect.com/journal/wao-journal

How to do a clinical trial? Recommendations from the aerobiological point of view

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ARTICLE INFO

Keywords:

Pollen data
Aerobiology
Allergen immunotherapy
Clinical trials
Checklist

ABSTRACT

Background: Allergy immunotherapy is still the only treatment of pollen allergy, providing a long-term effect. Clinical trials with pollen allergic patients are in need of validated, high quality pollen data and forecasts in order to grant comparability and to adhere to scientific standards. The aerobiological part of clinical trials remained hitherto not well defined, leaving the definition and use of pollen and forecast data more or less open.

Methods: Pollen data of eight Austrian pollen-monitoring stations were selected and used as an example to present a new method of pollen data replacement, in case of station failure. Gower's similarity provides an objective calculation based on a defined time frame and a specific aeroallergen (for example birch, grass, mugwort and ragweed).

Results: The ideal planning of the aerobiological part of a clinical trial with a pollen extract is described in detail with specific recommendations concerning site selection, pollen and forecast data, definition of the pollen season, and risk management. A checklist for every clinical trial with an aerobiological part was developed.

Conclusion: Virtual biogeographic regions are beneficial due to their objective establishment, and can be integrated into clinical trials. Pollen data is not the same as forecast data. Both datasets have to be critically evaluated by trained aerobiologists before they are used in clinical trials. Therefore, only institutions with aerobiological knowledge, at best ISO-certified, should be involved in clinical trials and handle the aerobiological tasks.

Background

Pollen allergies, as well as allergies in general, are a global health concern. They occur in a significant frequency as the example of allergic rhinitis (10–30% of the global population indicates).¹ Furthermore, pollen allergies are considered to still be on the rise and have a high socioeconomic impact.^{2,3} Allergies cannot be cured, but a number of treatments can relieve the symptoms. The therapy of pollen allergy relies on three important pillars: 1) specific allergen immunotherapy, 2) suppressing symptoms with medication, and 3) allergen avoidance.⁴ While the last measure, allergen avoidance, is focused on pollen information services, the specific allergy immunotherapy is plays not an important role in terms of support-oriented routines in aerobiology. There is no doubt that pollen forecasts are essential for and strongly requested by pollen allergy sufferers,^{4–6} and that this information has to be handled

with care and responsibility.⁷ However, aerobiology can provide more than guidelines in allergen avoidance. Clinical trials and specific allergy immunotherapies are in need of pollen information, pollen data, and aerobiological expertise.

Clinical trials and allergen immunotherapies (AITs) involve considerable effort and cost, and they have to show their effectiveness.⁸ However, allergen immunotherapies are still the only treatments that remedy the cause of the allergy.^{4,9,10} A recent review by the World Allergy Organization (WAO) formulated recommendations for the standardization of clinical trials.¹¹ Study design, patient selection, outcomes, statistics, as well as planning and performing a clinical trial, are covered in detail therein. It is interesting enough to note that aerobiological tasks were shortly commented on as well. Allergen exposure is recommended to be documented by using pollen data of the same method from the nearest pollen-monitoring station, and allocated evenly according to the patients'

Abbreviations: AIT, Allergy Immunotherapy; APIn, annual pollen index; EAACI, European Academy of Allergy and Clinical Immunology; EAN, European Aeroallergen Network (<https://ean.polleninfo.eu/Ean>); MPS, main pollen season; PHD, Patient's Hayfever Diary (www.pollendiary.com); SPIn, seasonal pollen index.

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<https://doi.org/10.1016/j.waojou.2019.100020>

Received 19 December 2018; Received in revised form 1 February 2019; Accepted 11 February 2019

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distribution.¹¹ However, detailed information and recommendations were not elaborated. More recent information on concepts and needs of clinical trials in allergen immunotherapy were summarized, but did not include more information on aerobiological issues besides the recommendation to use electronic health (e-health) and mobile health (m-health) technologies to capture individual exposure to allergens.¹² This leaves the aerobiological tasks of clinical trials more or less open to interpretation. We formulate herein a new standard for aerobiological tasks in clinical trials in order to increase the quality and comparability, to prevent any harm to persons involved, and to increase the success rates of clinical trials. Some AITs failed to prove the real efficiency of their testing agent because of an insufficient allergen exposure during the monitoring of the patients.¹³ This problem, as well as other causes such as different exposure to allergens (allergen exposure chamber versus natural exposure), are causes for negative clinical trials.¹⁰

The goals of this work are the: 1) development of criteria for aerobiological tasks, 2) importance of data quality, 3) the need for pollen data and pollen forecasts as well as relevant expertise, 4) establishment of risk management procedures, and 5) development of a checklist for clinical trials with aerobiological tasks. In short, we present here a new guideline of a “how to do” for all aerobiological issues before and during a clinical trial that includes pollen allergy patients.

Methods

Daily mean pollen concentrations from automatic volumetric pollen and spore traps of the Hirst design¹⁴ were evaluated following the minimum requirements.^{15,16} Calculations based on the pollen concentration data, such as the annual pollen index (APIn) or the seasonal pollen index (SPIn), follow the terminology in aerobiology,¹⁷ and are referred to whenever the terms *pollen data* or *pollen concentration* are used as follows and if not indicated differently. It should be noted that the term *pollen data* always refers to measured pollen concentrations in the air (pollen/m³ of air) on a scientific basis, whereas the term *pollen forecast* refers to predicted values.

All selected pollen-monitoring stations included in this study are located in Austria. Data were retrieved from the European Aeroallergen Network (EAN) database (www.ean.polleninfo.eu). The station code consists of the country designation (AT for Austria, first two letters) and the location designation (e.g. WIEN for Vienna, last four letters; [Tables 1 and 2](#)). Eight pollen-monitoring stations from the seven defined biogeographical regions of Austria were selected: Vienna (ATWIEN), Sankt Pölten (ATSTPO), Sankt Veit im Pongau (ATSVPG), Zell am See (ATZELL), Obergurgl (ATOBBER), Klagenfurt (ATKLAG), Feldkirch (ATFELD), and Allentsteig (ATALLE). Those sites were selected to reveal their similarities and dissimilarities, and are thus located in various areas. ATALLE is located in the northern part of Austria at a military base close to the border of Czech Republic, and is part of the biogeographical region “Bohemian massif” (region 1). ATSTPO is placed in the city of Sankt Pölten, the capital of lower Austria, and part of the biogeographical region “Danube valley and pre-Alpine areas” (region 2). The pollen-monitoring site ATWIEN is assigned to the biogeographical region of

Table 1

Geo-coordinates and heights above sea level of the eight Austrian pollen-monitoring stations, which were tested in the Gower's similarity matrix.

Pollen-monitoring station	Latitude	Longitude	Height above sea level (m)
Allentsteig (ATALLE)	48.69139	15.36722	596
Feldkirch (ATFELD)	47.23139	09.57972	507
Klagenfurt (ATKLAG)	46.63056	14.30556	446
Obergurgl (ATOBBER)	46.87861	11.01750	1930
Sankt Veit im Pongau (ATSVPG)	47.32639	13.14583	743
St. Pölten (ATSTPO)	48.21500	15.62667	265
Vienna (ATWIEN)	48.24889	16.35611	209
Zell am See (ATZELL)	47.33056	12.81278	764

the “Pannonian plains” (region 3), and located in the suburbs of Vienna representing the northeastern part of Austria. ATKLAG is located in Klagenfurt, the capital of the federate state of Carinthia, in the south-eastern part of Austria, and is assigned to the biogeographical region of the “Illyrian basins” (region 4). ATOBER is the pollen-monitoring station with the highest elevation in Austria and part of the biogeographical region “Northern limestone Alps” (region 5). ATSVPG and ATZELL are located in the small towns Sankt Veit im Pongau and Zell am See. Both towns are located in adjacent valleys of the central Alps, and are assigned to the biogeographical region “Alpine valleys central/south” (region 6). ATFELD is located in the city of Feldkirch in the western part of Austria, and assigned to the biogeographical region “Alpine valleys Austria west” (region 7). Details about geo-coordinates and heights above sea level are summarized in [Table 1](#).

The selected aeroallergens, namely pollen types, are birch (*Betula*), grasses (Poaceae), mugwort (*Artemisia*), and ragweed (*Ambrosia*), due to their importance in different phases of the pollen season in Austria. The similarity was calculated by the means of Gower's similarity.¹⁸ The following three criteria were chosen to define similarity: 1) the APIn for a total similar exposure, 2) the peak pollen concentration for a similar pattern within the pollen season, and 3) the pollen season start and end based on the EAN definition, starting with 1% and ending with 95% of the APIn for a similar time pattern of the exposure. The reference period included the last five years, 2013–2017, to put the calculation on solid ground and not only on one pollen season. Gower's similarity takes a range of distance and similarity measures into account. We adopted a non-weighted approach and therefore included all three categories in the same way. The results are summarized in [Table 2](#) for all four aeroallergens with 1.00 referring to perfect positive correlation. The next best result category is a significant correlation (0.70–1.00). Therefore, we defined herein a correlation coefficient above 0.70 as significantly similar enough to allow datasets to be used as replacement data.

The forecast models for showing the readiness to flower mentioned in the Results work with the maximum temperature in °C. The maximum temperature means both the actual temperatures measured and a temperature forecast, currently for up to 10 days. Negative values are skipped. As soon as the temperature reaches a certain sum (threshold), the readiness to flower is indicated. The model can be adapted depending on the region and the aeroallergen (pollen type), since these default settings have to be specified in advance. This and other procedures described in the results refer always to EAN data and work with routines programmed in the EAN database.

Results

The ideal procedure for the aerobiological part in a clinical trial or AIT is described as a result as follows, based on previous aerobiological routines and recently developed methods described herein.

Site and pollen data selection

The site selection is crucial and has to define the places of the study. These places have to fulfill certain criteria, but for the aerobiological perspective, the most important one is the exposure. Exposure must be high and relevant enough to allow a successful trial. This depends, of course, also on the selected aeroallergen. Most recommended sites will vary for a birch, grass, mugwort, or ragweed trial. Once the aeroallergens of interest are known, data from the last five to seven years should be reviewed. The average pollen season should be calculated with respect to the MPS (pollen season start, end, and peak) and its intensity (SPIn or APIn). The decision for certain countries and regions has to be based on these calculations to assure the exposure.

The site selection may then progress in more detail, namely the selection of single locations. Pollen-monitoring stations have to be allocated based on their proximity to possible locations. The density of a pollen-monitoring network will vary from country to country.

Table 2

Similarity matrix based on Gower's similarity for eight selected Austrian pollen-monitoring stations for four different aeroallergens: birch (*Betula*), grasses (*Poaceae*), mugwort (*Artemisia*), and ragweed (*Ambrosia*). Values with a high significant value of similarity of at least 0.70 are shown in bold, while values with a low similarity of 0.60 or below are shaded gray. Note that the level of similarity varies within the aeroallergen in focus.

<i>Betula</i>	ATWIEN	ATSTPO	ATSVPG	ATZELL	ATOBER	ATKLAG	ATFELD	ATALLE
ATWIEN	1.00							
ATSTPO	0.88	1.00						
ATSVPG	0.60	0.64	1.00					
ATZELL	0.54	0.54	0.89	1.00				
ATOBER	0.45	0.45	0.67	0.62	1.00			
ATKLAG	0.68	0.74	0.39	0.29	0.20	1.00		
ATFELD	0.90	0.80	0.70	0.60	0.51	0.58	1.00	
ATALLE	0.67	0.75	0.68	0.58	0.35	0.71	0.62	1.00
<i>Poaceae</i>								
ATWIEN	1.00							
ATSTPO	0.85	1.00						
ATSVPG	0.63	0.59	1.00					
ATZELL	0.75	0.82	0.70	1.00				
ATOBER	0.54	0.65	0.44	0.70	1.00			
ATKLAG	0.95	0.80	0.64	0.72	0.50	1.00		
ATFELD	0.57	0.63	0.70	0.75	0.58	0.52	1.00	
ATALLE	0.44	0.52	0.31	0.41	0.25	0.42	0.50	1.00
<i>Artemisia</i>								
ATWIEN	1.00							
ATSTPO	0.62	1.00						
ATSVPG	0.42	0.46	1.00					
ATZELL	0.59	0.63	0.76	1.00				
ATOBER	0.63	0.67	0.76	0.97	1.00			
ATKLAG	0.62	0.87	0.33	0.50	0.54	1.00		
ATFELD	0.49	0.86	0.52	0.73	0.76	0.77	1.00	
ATALLE	0.82	0.50	0.24	0.41	0.44	0.50	0.37	1.00
<i>Ambrosia</i>								
ATWIEN	1.00							
ATSTPO	0.76	1.00						
ATSVPG	0.47	0.70	1.00					
ATZELL	0.08	0.32	0.61	1.00				
ATOBER	0.42	0.65	0.90	0.66	1.00			
ATKLAG	0.66	0.89	0.81	0.43	0.77	1.00		
ATFELD	0.58	0.80	0.89	0.50	0.84	0.89	1.00	
ATALLE	0.83	0.77	0.47	0.08	0.42	0.66	0.56	1.00

Therefore, more or fewer options will become available. Although it is known that pollen measurements are point measurements, research indicates they are valid in general for an area within 30 km reach (e.g. Ref. 19). This range will depend on the local geography, e.g. it will be smaller in mountainous areas and higher for plains. In addition, the pollen-monitoring station should be on about the same elevation within the same biogeographic region, besides the proximity to the study center.

Biogeographic regions are defined based on geography and vegetation, and should summarize an area of similar exposure and pollination patterns. The advantages of real biogeographic regions are having several pollen-monitoring stations in the same region and thus allowing easy replacement in the best case, and similar, providing a known framework for pollen allergy sufferers (e.g. vegetation, climate). The disadvantages of real biogeographic regions are low replacement options in less dense pollen-monitoring networks and eventual notably different conditions for pollination (e.g. elevation, mountainous regions). Still, the biogeographic regions should be considered and taken into account in the first place (see virtual biogeographic regions in Risk Management and alternative options, Table 2).

Based on our experience, pollen data has to be reviewed for the last five years regarding its usefulness and completeness for a clinical trial. Pollen data should be as complete as possible. Pollen-monitoring stations with regular gaps within the MPS or the peak days over the last years should be excluded. The availability of pollen data has to be checked as well. EAN suppliers comprise all kinds of institutions (universities, charities) and persons (private persons, scientists). Data may not be available for some of the pollen-monitoring stations or may have to be requested separately. The quality of pollen data has to be evaluated in order to grant high quality for clinical trials. These quality checks have to be performed by trained aerobiologists and include all possible data

flaws, including errors in date, typing, transfer, and identification. The frequency of data delivery can be an important point if recent pollen data is needed during the trial and not only retrospectively. It has to be clarified beforehand as to which time frames are acceptable. There are pollen-monitoring stations of exquisite quality but with a huge time lag of delivery.

Season definition and forecast data

The selection of the pollen season definition is a crucial decision as it influences the time period under study and thus the results. Therefore, the defined period under study could impact the aerobiological result²⁰ as well as results based on symptom data.²¹ There are two different methods to define the pollen season start and end: consider a certain threshold of pollen concentrations or consider a percentage.²² The disadvantage of methods using the percentage is a longer exposure time (useful for aerobiological routines) and its applicability as exclusively retrospective; therefore, this method is not readily valid for clinical trials. In contrast, methods using a certain threshold are ideal and can be used throughout the pollen season.²² Recently, new definitions were developed in an EAACI (European Academy of Allergy and Clinical Immunology) taskforce for the birch, grass, ragweed, cypress, and olive pollen season.²³ These pollen season definitions were already tested and proved their usefulness.^{24,25} In any case, the pollen season definition used and its method have to be chosen with care and be clearly stated.

Pollen forecasts have to be consulted before and throughout the clinical trial to be able to react on short-term changes and to optimize the study plan. Moreover, they have to be included before the study plan is finalized. Three different pollen forecasts that should be considered are: 1) long-term, 2) mid-term, and 3) short-term. Long-term pollen forecasts

support the outline and rough time table for the clinical trial. This forecast tries to indicate the probable time period of exposure to the aeroallergen selected. Additionally, this forecast is prepared with standard statistics: start day, end day, peak day, peak pollen concentration, and APIn are calculated as the average of the last five years at minimum. The average gives a good estimation of the usual situation, and this data is used right away except for the start day. We recommend using the start day modified with 10 days earlier because of the anticipation effect.²³ The mid-term forecasts give more indication of the start of the pollen season in focus. This forecast uses a model for the readiness to flower for the respective aeroallergen (see Methods). The date for the readiness to flower is not imperatively the start day. Instead, it is the day where the plant reaches its capability to flower. Pollination will then start if weather conditions are favorable. Short-term pollen forecasts are needed in a clinical trial to react to the ongoing pollen season, may it be an unusual situation (early/late start) or weather-related events within the MPS (delay of a peak). These short-term pollen forecasts consist of a short-term model for the readiness to flower and various pollen distribution models such as COSMO-ART²⁶ or SILAM,²⁷ and should consult the expertise of local pollen information services whenever possible.

Risk management and alternative options

A detailed preparation of a clinical trial as described before, including the careful selection of sites, pollen-monitoring stations, and pollen forecasts, does not prevent possible obstacles. Therefore, risk management including a disaster plan has to be employed. The most frequent obstacle is pollen-monitoring station failure. Therefore, pollen data has to be gathered from an adequate but different location. The site selection includes the selection of other possible pollen-monitoring stations in descending order, and of the same biogeographic region. There is another option if this approach should fail. Therein, similarity can be calculated and an adequate station for a specific aeroallergen can be found in virtual biogeographic regions (see below). Risk management should take care of these and other possible issues.

Institutions handling the aerobiological part in clinical trials have to prove their aerobiological expertise in order to provide and process pollen and forecast data. Moreover, they have to be ISO-certified (International Organization for Standardization) or prove their expertise in a comparable way. These prerequisites are necessary since any data issue (e.g. low quality and/or completeness) may harm the concerned

persons⁷; this is especially true in clinical trials. The resulting checklist of these findings can be used for the described procedures in order to consider all possible issues (Table 3).

In addition, we described herein for the first time a possible way to find similar pollen data in case of pollen-monitoring site failure. Gower's similarity shows possible replacement of pollen-monitoring stations (Table 2). If data for ATWIEN are missing, ATSTPO could be used instead for birch, grasses, and ragweed, but not for mugwort. However, other pollen-monitoring stations achieve even higher similarity with ATWIEN, although they are in different biogeographical regions, for example ATFELD for birch, ATKLAG for grasses, and ATALLE for ragweed. The most similar dataset should be chosen of course, and this depends on the aeroallergen as shown here. Thus, a virtual biogeographic region can be defined by the means of Gower's similarity, depending on the aeroallergen and possibly the time period in addition to real biogeographic regions and their possible disadvantages.

Discussion

Although clinical trials are in need for both current pollen data and forecasts, it should be emphasized that the two terms designate two different datasets, which must never be mixed. *Pollen data* means pollen concentrations in the air (pollen/m³ of air) measured on a scientific basis (see Methods), whereas *pollen forecasts* are predicted values which may dramatically deviate from the measured values depending on the forecast quality.²⁸ Therefore, pollen forecast data must never be used in place of pollen data except for one special case: if the risk management fails due to a pollen-monitoring station failure and neither the real nor the virtual biogeographic region could provide an adequate replacement dataset, the disaster plan may rely on forecast data. The forecast data has to originate from a trustworthy institution capable of pollen-forecasting to avoid major data issues.^{7,28} In addition, it has to be reviewed to determine if it is suitable and accurate enough. However, this scenario is highly unlikely.

Recommendations are limited to pollen data gained by Hirst-type volumetric pollen stations and to the routine aerobiological analyses following the minimum recommendations.¹⁵ This data is robust, standardized, and tested in various ways by the aerobiological community. Currently, it is doubtful if and how other data types are obtained, e.g. by automated pollen samplers that can be used for such applications. Those, however, are still in a test phase^{29–31} and have to solve a range of pitfalls,

Table 3

Recommended checklist for the aerobiological part in AITs and clinical trials. All issues have to be addressed and considered during the planning of a trial in order to grant success and higher comparability of such studies.

Checklist categories	Checklist points
Site selection	<ul style="list-style-type: none"> • Exposure is relevant to the selected aeroallergen in the concerned country/region • Exposure to the relevant selected aeroallergen is not significantly overlapped by another aeroallergen of importance • Impact of cross-reactivity of other pollen types has to be considered
Pollen season definition	<ul style="list-style-type: none"> • Pollen season definition is clearly described • Pollen season definition is applicable to the chosen site/region • Pollen season definition considers local/regional peculiarities, including possible pollen season overlaps • Pollen season definition captures the MPS
Pollen concentration data	<ul style="list-style-type: none"> • Pollen concentration data is available • Pollen concentration data is complete or sufficiently available • Pollen concentration data is of good quality • Pollen concentration data is frequently delivered throughout the season, at least enough for the needs of the clinical trial • Pollen concentration data is real measured data of Hirst-type volumetric pollen and spore traps evaluated following minimum recommendations (no forecast data and no other data types previously not validated) • Pollen data is available and applicable (as described above) for a replacement station if needed
Pollen forecast data	<ul style="list-style-type: none"> • Short-term pollen forecasts are available and of good quality • Mid-term pollen forecasts are available and of good quality • Long-term pollen forecasts are available and of good quality
Risk management	<ul style="list-style-type: none"> • Disaster plan is existing
Institutions involved	<ul style="list-style-type: none"> • Pollen concentration data is available in similar pattern in case of pollen-monitoring station failure for a real and/or virtual biogeographic region • Institutions involved in providing aerobiological expertise and data are capable of these tasks • Institutions involved are ISO-certified

such as comparability, range of pollen types assessed, and precision rates, among others.⁷

The EAACI pollen season definitions²³ are highly recommended, because they were developed for clinical trials based on the most recent findings and represented a new standard and agreement of the scientific community. They could even be applied to other tree and weed taxa, such as hazel (*Corylus*), alder (*Alnus*), and mugwort (*Artemisia*), and catch more likely the MPS with the highest exposure, which is recommended for clinical trials.²²

The forward shift of ten days for the long-term pollen forecast at the start of the pollen season is a safety measure necessary for a clinical trial in order not to begin too late, and is explained as follows. Anticipation effects are observed for several locations and different pollen seasons, and show the reaction of pollen allergy sufferers before relevant pollen concentrations are in the air.²³ There is more than one plausible reason for this phenomenon, although it has not been clarified until now. Following reasons could explain this outcome: 1) pollen concentrations are point measurements and could miss very local exposure, as known for *Alnus x spaethii*,³² 2) cross-reactivity, 3) pollen information preparing the population for exposure soon and psychological reasons, 4) lower thresholds after a period of relief (all discussed in Ref. 23), and 5) allergen release apart from pollen release^{33–36} and/or connections with air pollution including increased allergenicity, inflammatory effects, and interaction with microscopic particles.^{37–39}

A future aspect of aerobiological data in clinical trials is the virtual biogeographic region, which was presented herein as an example for eight different Austrian pollen-monitoring stations. The advantages of this approach are the independence from national networks, pollen-monitoring stations do not have to be very closely situated, and data replacements are based on an objective calculation of similarity. The example of this study shows that ATSTPO, which is the closest pollen-monitoring station to ATWIEN, would be a good replacement for birch and grass pollen data. However, stations from other parts of Austria could even be a better replacement for this purpose (e.g. ATFELD for birch pollen or ATKLAG for grasses). Although ATSTPO has a closer Euclidian distance than ATALLE, the latter shows a stronger similarity regarding the weed taxa of mugwort and ragweed; hence, it is a better replacement option than the ATSTPO station, which is nearer in distance.

Only institutions capable of taking over aerobiological tasks should be entrusted with such tasks. ISO-certification and/or proven expertise on the relevant tasks should be the prerequisites for providing data and expertise for clinical trials and AITs. Documentation of all processes is a crucial point, through adherence to standard operation procedures (SOPs). It should also include the storage of pollen data in an appropriate way. The slides themselves or the scans of microscopic images could be stored to serve as proof for 20 years or more. A future outlook should certainly include digital documentation. EAN as a central point for a huge data pool with additional data quality routines and aerobiological expertise could serve in Europe as a partner to handle such support and services. It would be advantageous to build up similar centers on other continents and to connect these centers for a global network in order to enable clinical trials and AITs of the same high quality.

Summary and Conclusions

Clinical trials focusing especially on pollen allergy, including AITs, are in special need for aerobiological expertise and have to consider specific issues in order to grant the success of such trials, since one of the European Medicine Agency (EMA) claims is the proven efficacy in the first pollen season.⁴⁰ Among them, the site selection, pollen data, forecast data, selection of the pollen season definition, and risk management in case of pollen-monitoring station failure or data gaps all have to be carefully considered. Certain methods as presented herein can facilitate objective data assessment, e.g. by searching for similar data for an aeroallergen and using virtual biogeographic regions.

Consider the following points to ensure a satisfactory level of the aerobiological part:

- 1) Is the exposure to the selected aeroallergen high enough in the chosen site/region/country, or are there possible issues due to overlapping exposure to other pollen taxa?
- 2) Is pollen data available, most complete, of high quality including the methodology, and frequently delivered for the site chosen, and is there a replacement if needed?
- 3) Is the selected pollen season definition adequate for the purpose and applicable for the site chosen?
- 4) Are short-, mid-, and long-term forecasts available for the site chosen?
- 5) Is there a risk management plan (data gaps, pollen-monitoring site failure, and others)?
- 6) Are the institutions involved (at best, ISO-certified) in the aerobiological part of the study capable of evaluating and processing such data?

We recommend following the developed checklist herein to increase the quality of AITs and clinical trials, and to grant better comparability between such trials.

Declarations

Competing interests

All authors report no competing interests.

Author contributions

KB drafted the main body of the manuscript, supervised the analyses, and prepared the Tables. MB performed the analyses and prepared the Tables. KCB supported with medical advice and supervised the drafting of the manuscript. UB supervised the study and initiated the idea of virtual biogeographical regions. All authors took part in writing the manuscript and approved the final version of the manuscript.

Ethics approval and consent to participate

No ethics approval was needed.

Consent for publication

All authors confirm their consent for publication.

Availability of data and material

Users were guaranteed anonymity; thus no raw data or individual user data is available.

Funding

No funding was received for this study. However, the company Bencard/Allergy Therapeutics[®] financed the publication fee.

Acknowledgments

We owe our thanks to all EAN data suppliers who build the fundament for such studies and clinical trials. The authors want to thank especially the data suppliers Sabine Kottik (ATALLE, ATSTPO, and some years of ATFELD), Notburga Oegg-Wahlmüller (ATOBBER), Helmut Zwander (ATKLAG), Margit Langanger (parts of ATZELL), Ulrike Gartner (ATSVPG), and Ulrike Langmann (parts of ATZELL) as well as the local pollen information services of Tyrol, Carinthia, and Salzburg for providing the data of their pollen-monitoring stations for the Gower's similarity matrix, in addition to already available data from ATWIEN and

ATFELD provided by the author team. In addition, we want to thank Christoph Jäger for his constant efforts with the EAN database. We are grateful to two anonymous reviewers who improved the manuscript with their constructive reviews. We are grateful to the company Bencard/Allergy Therapeutics[®] for financing the publication fee.

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