Human exposure to airborne pollen and relationships with symptoms and immune responses: Indoors versus outdoors, circadian patterns and meteorological effects in alpine and urban environments

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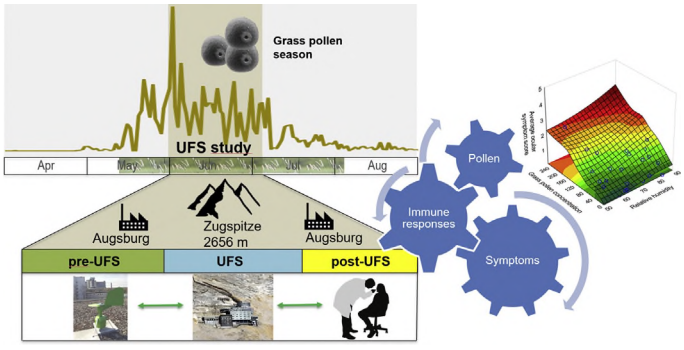
HIGHLIGHTS

- Pollen concentrations and symptoms were monitored in urban vs alpine ecosystem
- Higher pollen exposure led to higher severity of symptoms
- Staying in an alpine environment lowered allergic symptoms and immune responses
- Nasal or pulmonary symptoms and immune responses were retained low for 2 weeks
- Relative humidity >60% lowers to half the threshold of pollen triggering symptoms

GRAPHICAL ABSTRACT



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1. Introduction

Clinical evidence reveals a general increase in both the incidence and the prevalence of respiratory allergies, including allergic rhinitis and asthma (e.g. Bunne et al., 2017; Pawankar, 2014). According to The World Allergy Organization estimates (Pawankar et al., 2013), allergic rhinitis is currently affecting up to 30% of the population. This percentage varies among cities, countries and continents because of environmental and other factors and can even exceed 40% (e.g. Morais-Almeida et al., 2013; Sibbald and Strachen, 1995). Hence, allergies are a major public health problem that has worsened in recent decades and it is now recognised as a major global epidemic, also with considerable economic burden (Linneberg, 2016; Ring et al., 2014).

Climate change, air pollution and urbanisation could indirectly favour respiratory allergies, as increasing temperatures bring about earlier flowering and pollination periods and concomitantly overall shorter allergen-free seasons (D'Amato et al., 2015; Fotiou et al., 2011; Schiavoni et al., 2017; Ziello et al., 2012; Ziska et al., 2003). Long-term health impacts may be related not only to air pollution and changes in lifestyle, but also to an actual increase in the amount of airborne allergenic pollen (e.g. Fotiou et al., 2011; Ziello et al., 2012). Although local trends may vary greatly, climate change has already resulted in significant increases in the vegetation coverage or abundance of several pollen taxa, such as *Ambrosia artemisiifolia* in the USA and parts of Europe, especially in north Italy and on the Pannonian plain (e.g. Lake et al., 2017; Sikoparija et al., 2017; Storkey et al., 2014; Ziello et al., 2012; Ziska et al., 2011).

Pollen allergy can manifest itself as allergic rhinitis, allergic conjunctivitis and/or allergic bronchial asthma (e.g. Erbas et al., 2018). International literature identifies grass pollen as the leading aeroallergen worldwide (e.g. García-Mozo, 2017; Weeke and Spieksma, 1991; Wu et al., 1999). Allergenic grasses consist of both annual and perennial species, many of which are highly cosmopolitan and, hence, they are found in a wide variety of latitudes and biogeographical regions and in natural as well as urban habitats (e.g. Pignatti, 1982; Lewis et al., 1983). According to epidemiological and clinical studies across the globe, sensitisation rates to grass pollen can reach up to 80% of the total atopic population (e.g. Belver et al., 2007; Erbas et al., 2018; Kobzar, 1999; Wu et al., 1999).

What is currently lacking, however, is information on the real-life health impacts of pollen exposure and climate variability on the allergic population. Even though there have been recent attempts to elucidate this relationship from existing respiratory symptoms' databases (e.g. Karatzas et al., 2014), there is still a significant knowledge gap. It is still not clear whether exposure to allergenic pollen induces symptoms in a direct and immediate way, what kind of symptoms it induces (ocular, nasal, pulmonary or combinations) and if the symptoms vary in severity depending on exposure-related behaviour and duration of the exposure. Also, it has never been documented whether symptoms can also be observed in non-atopic people. Moreover, to our knowledge, none of the above has been examined under differing environmental conditions (urban versus natural environment) or in extreme environments (e.g. high altitude). Finally, given that more 80% of our time is spent indoors (Klepeis et al., 2001), no conclusion has been drawn whether the indoor or the outdoor pollen load (where also pollen is mostly monitored worldwide) are most relevant for predicting the genuine human exposure and the resulting respiratory symptoms.

Moreover, there is little information on the kinetics between exposure and reaction, i.e. if the relationship between pollen exposure and symptoms is linear or non-linear, if it varies depending on the duration of pollen exposure, or if there is time lag between the actual pollen exposure and the occurrence of allergic symptoms. The above questions make pollen season forecasting (and consequent symptom forecasting) rather complex, thus highlighting the need for additional research so as to achieve accurate and operational predictive models, which comprises one of the first line allergy management tools.

The aim of this study was, therefore, to assess how short-term changes in pollen exposure translate into changes in respiratory symptoms and nasal immune responses. To achieve this, we had to assess the symptom-related genuine exposure, by monitoring symptoms in two well-characterised cohorts of non-allergic and pollen allergic subjects and in two different pollen exposure regimes, a high pollen one in an urban ecosystem and a low pollen one in an alpine, high-altitude ecosystem. During peak grass pollen season, the subjects were transferred from an urban environment with high airborne pollen load to a natural, high-altitude, low pollen environment, and back again after a 12-day stay. The questions we asked were: What effect does lower pollen exposure have on pollen allergic symptoms and immune responses and how can we quantify this? And how long lasting is the potential health benefit and what are the environmental factors affecting the pollen-symptoms interaction?

2. Material and methods

2.1. Study design and locations

The study lasted from 1 June to 6 July 2016. The first 12-day interval, from 1 June to 13 June, took place in the region of Augsburg. On 13 June, all participants met at the railway station of the city of Garmisch-Partenkirchen, situated on the foothills of Zugspitze mountain in the Bavarian Alps, and jointly travelled by cog railroad up to the Schneefernerhaus [UFS (Umweltforschungsstation Schneefernerhaus)], an environmental research station situated some 300 m below the summit of Zugspitze mountain (elevation 2656 m), where they stayed without interruption until 24 June (for a total of 12 days). During the whole stay, daily habits were recorded on an 8-hourly scale, i.e. hours spent outdoors versus indoors and hours spent on exercising, either indoors or outdoors. On 24 June, all participants collectively left the UFS and travelled back to their homes. The final 12-day study interval, again in the Augsburg region, ended on 6 July 2016.

2.2. Pollen monitoring

Grass pollen was examined in 2016 for both sites, UFS and Augsburg. This pollen taxon was selected because it is the most important outdoor aeroallergen and common in most environmental regimes across the world (e.g. García-Mozo, 2017). Biomonitoring took place at ground level, using Hirst-type volumetric traps (Burkard Manufacturing Co. Limited, Rickmansworth, Hertfordshire, England, UK) (Hirst, 1952). Grass pollen was identified (at the family level Poaceae) under light

microscope and grains were counted per cubic metre of air, on two time resolutions, per day and per 8 h, throughout the whole study (total duration of 36 days). The biomonitoring techniques used (details in Section 2.2.1) are typical for pollen data collection, followed by most scientists (e.g. British Aerobiology Federation, 1995).

2.2.1. Pollen monitoring in Augsburg

Airborne pollen in the city of Augsburg was collected by use of a 7-day recording Burkard volumetric trap located at the Bavarian Environmental Agency bureau, at ground level. The trap was equipped with a vacuum pump drawing 10 l of air min^{-1} through a narrow orifice. Air particles were trapped on an adhesive-coated (Burkard gelvatol) transparent plastic tape (Melinex), supported on a clockwork-driven drum, which moved at a speed of 2 mm h^{-1} making a complete revolution in one week. The tape was then removed and cut in seven equal sections, each representing a day of sampling (viz. of 48 mm of tape per day). The tape sections were stained with a solution of saffranine, gelatine, glycerol and phenol and were mounted on microscope slides, each slide representing a 24 h period. Grass pollen grains were counted in 12 transverse traverses per slide, each transect representing a 2-hourly interval, under a light microscope (Leica DM750) at a magnification of ×400. Counts were made on a bi-hourly basis and expressed as mean daily pollen concentrations (number of pollen grains per m^3 of air d^{-1}) or mean 8-hourly pollen concentrations, investigating for differences among morning (06:00–14:00), afternoon (14:00–22:00) and night (22:00–06:00) (so as to be comparable to the symptom registry time resolution).

2.2.2. Pollen monitoring on the UFS

On the UFS, pollen monitoring was performed using portable Burkard samplers. Sampling was conducted every 8 h (morning, afternoon, night) and lasting for half an hour each time. Two portable samplers operated at the same time, both indoors and outdoors. The laboratory techniques including pollen identification and counting and the measurement units used were exactly the same as for the stationary devices described in Section 2.2.1.

2.3. Human cohort characteristics

Healthy non-allergic and grass pollen allergic volunteers were recruited in the Augsburg region from February to May 2016. Candidates underwent an initial screening procedure to exclude perennial rhinitis, nasal polyps or chronic rhinosinusitis, including a blood test for IgE measurement. An initial cohort of 10 + 10 allergic and healthy participants was recruited. Based on the performed screening but also on the consistency and reliability of their participation (i.e. continuous presence in the required study sites, and regular registering of symptoms), finally six healthy, non-allergic volunteers and five pollen allergic (otherwise healthy) patients with self-reported symptoms during the grass pollen season and CAP class ≥2 for grass pollen were included in the study. Healthy non-allergic volunteers had overall low total serum IgE levels (19.0 ± 8.1 IU/ml; mean ± SEM) and no specific IgE (<0.03 IU/ml) against any seasonal or perennial aeroallergen, as tested by ImmunoCAP and ISAC (Phadia/Thermo Fisher). Allergic rhinitis patients included in the study had elevated total serum IgE (141.4 ± 70.1; mean ± SEM) and elevated grass pollen-specific IgE levels (average CAP class 3), without co-sensitisation against house dust mite. For an overview of participants' characteristics, see also Table 1. Sensitisations were additionally assessed by component-resolved IgE diagnostics (ISAC aeroallergen chip, Thermo Fisher; data not shown). The study was approved by the local ethics committee (code: 19/15) and conformed to the guidelines of Helsinki. Study participants were enrolled after written informed consent.

2.4. Determination of immunoglobulins, cytokines and chemokines in nasal samples

A total of 9 nasal secretions were collected per subject throughout the study (as in Gilles-Stein et al., 2016). Briefly, a strip of absorbent filter paper (Pall, Leucosorb) was inserted ipsilaterally into the nostril and kept there for 45 s. The filter paper strip was then placed into the insert of a 1.5 ml spinning filter tube (Costar). Secretion fluid was extracted by adding 100 μl of double-distilled water to the paper strip and spinning it down in a pre-cooled centrifuge (4 °C) for 5 min at 10,000 ×*g*. Nasal secretion weights were assessed by weighing the tube plus filter paper before and after sample collection. Local cytokine release was calculated by normalising cytokine concentration to nasal secretion volume.

Chemokines, cytokines and immunoglobulins were measured in nasal secretions via multiplex magnetic bead-based detection kits (Bio-Plex Pro Human Isotyping Panel 6-plex for IgA, IgM, IgG_1, IgG_2, IgG_3 and IgG_4; Human IgE Isotyping Assay for IgE and a custom 9-plex for IL-33, CCL24/Eotaxin-2, CCL4/MIP-1β, CCL2/MCP-1, CCL22/MDC, CXCL8/IL-8, IL-16, G-CSF and IL-1β) according to the manufacturer's instructions. Optimal sample dilutions were examined beforehand. Nasal

Table 1
Overview over characteristics of study participants.
Participants in the study, their age and gender and the initial screening results [serum total IgE and specific IgE against a set of common aeroallergens (perennial and seasonal) (by ImmunoCAP)].

				Perennial allergens		Pollen allergens				
Subject ID	Gender (m/f)	Age (years)	Total IgE (IU/ml)	HDM (IU/ml)	Cat dander (IU/ml)	Timothy grass (IU/ml)	Rye (IU/ml)	Birch (IU/ml)	Hazel (IU/ml)	Mugwort (IU/ml)
Allergic										
A1	f	57	60.4	0.02	0.01	10.70	7.10	0.15	0.22	0.27
A2	f	33	335.0	0.19	44.70 *	19.00	8.57	0.11	0.05	0.20
A3	m	20	19.3	0.03	0.00	0.83	0.47	0.10	0.02	0.01
A4	f	20	266.0	0.03	0.05	94.30	61.30	0.07	0.03	0.65
A5	f	32	19.5	0.00	0.00	2.53	1.85	0.00	0.00	0.02
Non-allergic										
NA1	f	28	93.4 **	0.02	0.00	0.02	0.03	0.01	0.01	0.01
NA2	m	29	7.7	0.01	0.00	0.02	0.02	0.00	0.00	0.00
NA3	f	25	29.9	0.00	0.00	0.00	0.00	0.00	0.00	0.00
NA4	m	65	14.0	0.01	0.00	0.01	0.01	0.00	0.00	0.00
NA5	f	63	8.7	0.01	0.00	0.01	0.01	0.00	0.00	0.00
NA6	m	26	7.8	0.00	0.00	0.00	0.01	0.00	0.00	0.00

*Participant was not exposed to cats during the study. **Participant was sensitized against bee and wasp venom (data now shown), hence the high total IgE value.

samples, standards and controls were analysed via Bio-Plex 200 System (Bio-Rad Laboratories) with control and analysis software Bio-Plex Manager 6.1 (Bio-Rad Laboratories). Standard curves for each target were calculated to determine the concentration of immune mediators.

2.5. Monitoring of symptoms

Throughout the study, participants filled in a questionnaire daily on their smartphones or laptop computers, covering questions on general wellbeing, medication use and allergic symptoms. Symptoms included nasal, ocular and pulmonary symptoms, with severity ranging from 0 to 3 (0: none, 1: mild, 2: moderate, 3: severe). Participants were also asked about the time of day their symptoms occurred, as specified in 8-hour intervals (morning: 6–14 h, afternoon: 14–22 h, night: 22–6 h). Additionally, the questionnaire contained questions on exposure-relevant behaviour, e.g. how many hours they had spent outdoors and when exactly or whether they had engaged in outdoor activities that predispose to potentially high pollen exposure, such as gardening, lawn mowing and outdoor sports, if they kept the windows open at night or if the participants had washed their hair before going to sleep.

2.6. Meteorological data

Meteorological data (air temperature, precipitation and relative humidity) were obtained for Zugspitze and Augsburg for the respective time-periods from the open access database of the German Weather Service (DWD Climate Data Center, 2018).

2.7. Data analysis

All data were examined at two different timescales, per day and per 8-hourly intervals. Differences among sites (before UFS, during UFS, after UFS) and time intervals (morning, afternoon, night) were investigated in all possible combinations and interactions (*t*-test for dependent samples, one-way, nested and full factorial ANOVA, 2-degree factorial ANCOVA). Moreover, Pearson correlations, and one-way, multiple and full factorial regressions were performed, along with time series analysis (cross-correlations), so as to examine the relationships of symptoms versus all other co-factors. All analyses were examined at the significance level of $p = 0.05$. Differences were corrected after Bonferroni criterion and homogenous groups were identified in all cases. In the regressions, the Least Squares Distance fitting was adopted with a stiffness of 0.2, so as to detect local data peculiarities. In all factorial analysis (ANCOVA, regressions), the stepwise backward elimination method was applied, so as to determine which the main co-factors are for the optimum forecasting model. All data analyses were carried out in Statistica 13.

3. Results

3.1. Time course of symptoms related to pollen exposure

In the first study interval (pre-UFS), which coincided with the peak of grass pollen season in Augsburg, airborne grass pollen concentrations reached up to 242 pollen grains/m^3 (average of 87 pollen grains/m^3). During this time, mean symptom scores in non-allergic participants were low, whereas they were high in the allergic cohort. Peaks in symptoms of allergic patients coincided with peaks in pollen concentrations. In the second study interval on UFS, airborne grass pollen concentrations were low, reaching no more than 73 pollen grains/m^3 (average of 18 pollen grains/m^3), and, likewise, symptoms were low. In the third interval, again in Augsburg, grass pollen counts were high again, but somewhat lower than during the first interval. In line with this, symptoms rose again but remained lower than before the UFS stay (Fig. 1). Surprisingly, in the non-allergic cohort, (nasal) symptoms were observed throughout the study and regardless of the site and time interval.

3.2. Site-specific differences in pollen exposure

Pollen exposure was found to be significantly higher outdoors compared to indoors: outdoor grass pollen concentrations were up to 17 times higher than those measured indoors (Fig. 2A). In contrast, we found no significant differences depending on the time interval of pollen sampling (day, afternoon, nighttime pollen concentrations) on the UFS: pollen was present homogenously throughout the day. When comparing pollen concentrations for each site separately, though, we found that in Augsburg (and particularly in the first study period), pollen concentrations were significantly higher in the morning and afternoon compared to those during night (Fig. 2B) and especially as compared to the UFS.

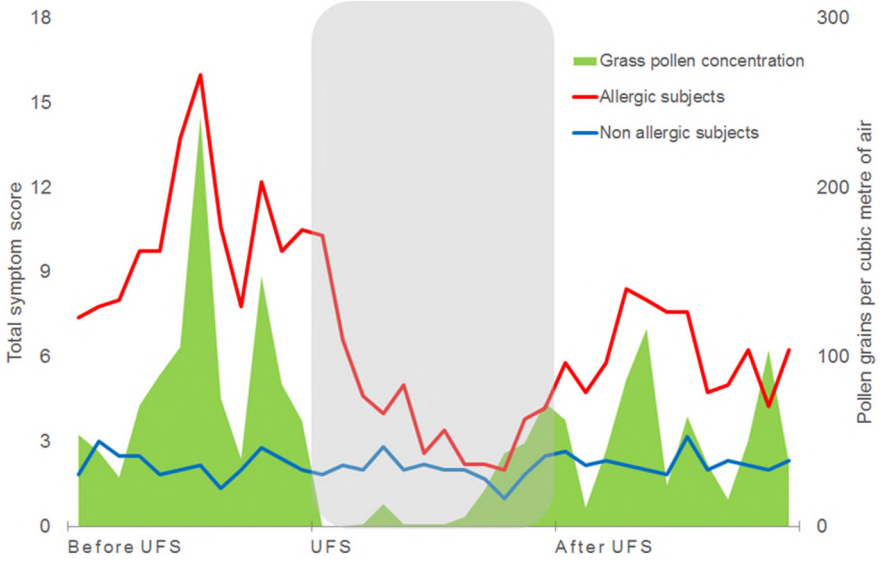

Fig. 1. Time course of daily total symptom scores in relation to pollen concentrations. Total symptom score of pollen-allergic patients and non-allergic subjects vs. airborne grass pollen concentrations over time (n = 36 days). The shaded area marks the UFS stay. Before/after UFS: City of Augsburg. UFS: Zugspitze mountain.

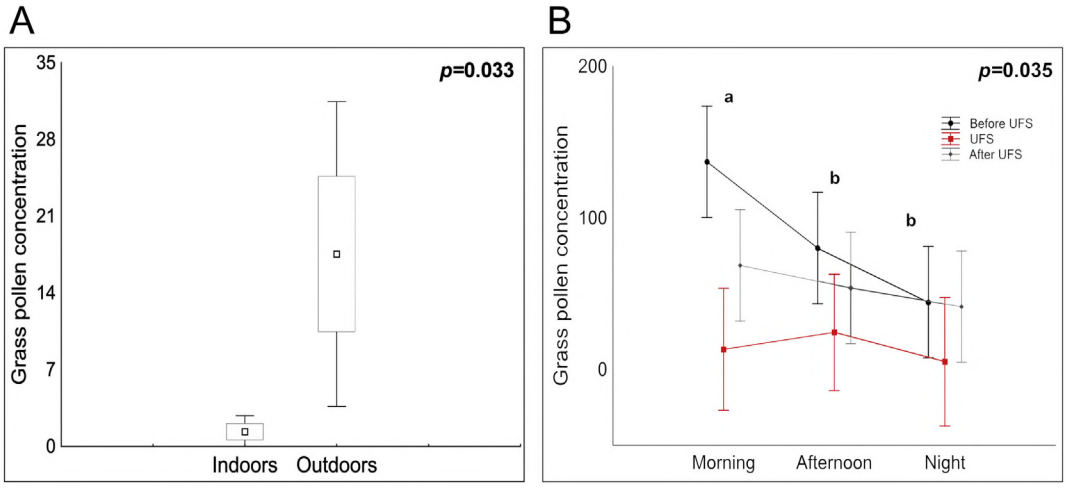

Fig. 2. Differences in airborne grass pollen concentrations among study sites, dependent on outdoor vs. indoor sampling and sampling time per day. A. Spatial differences: pollen indoors vs. outdoors on the UFS (*t*-test for dependent samples: central marker stands for the average, box for the standard error and bars for standard deviation); B. Temporal differences: outdoor pollen exposure comparison among morning vs. afternoon vs. night and between UFS vs. Augsburg (nested ANOVA: outdoor pollen concentration was the dependent variable, time interval (nested parameter) and site the categorical predictors). a, b: significant differences after Bonferroni correction (a > b). Significance level *p* is indicated.

3.3. Nasal immunoglobulin responses to different exposure regimes

To examine whether the UFS stay had an influence on the nasal immune response of grass pollen allergic patients, we determined levels of total nasal immunoglobulins as well proinflammatory cytokines and chemokines before, during and after the UFS stay, and correlated the results with the study interval (before, during or after UFS), including airborne pollen concentrations as covariate. It was found that total nasal IgE- (Fig. 3A) as well as nasal IgM levels (Fig. 3B) were significantly lower on UFS and after UFS as compared to before UFS. The other immunoglobulins did not differ between intervals in this model (Fig. 3C–G).

3.4. Nasal cytokine- und chemokine responses to different exposure regimes

Levels of cytokines and chemokines in nasal secretions were found to differ between pre-, during and post-UFS, with most of the nasal cytokines studied decreasing during the UFS stay, as for IL-33 (Fig. 4A), CCL24/Eotaxin-2 (Fig. 4B), CCL4/MIP-1β (Fig. 4C), CCL2/MCP-1 (Fig. 4D) and CXCL8/IL-8 (Fig. 4F). These were found to differ significantly between study intervals, being lowered on UFS and not statistically altering and staying decreased for the whole post-UFS period. CCL22/MDC, IL-16, G-CSF and IL-1β (Fig. 4E, G, H and I, respectively) did not differ significantly between study intervals.

3.5. Symptoms in response to pollen exposure levels and environmental factors

To assess the relationship between pollen concentrations and symptoms, we first performed time series analysis (cross-correlation) of daily symptoms versus airborne pollen concentrations. In the non-allergic cohort there was no significant correlation of any type of symptoms with airborne grass pollen concentrations ($p > 0.05$) and regardless of the site under examination. In contrast, a significant cross-correlation was observed with all forms of symptoms with airborne grass pollen in the grass pollen-allergic cohort ($p < 0.01$). There was a significant lag effect of ocular and pulmonary symptoms with pollen concentration of up to the previous day and up to 3 days before for nasal symptoms. The strongest cross-correlation was observed on the same date of pollen occurrence and symptom manifestation (lag = 0) and for all forms of symptoms, with the ocular symptoms exhibiting a stronger and more immediate effect ($r = 0.71$), compared to nasal ($r = 0.53$) and pulmonary symptoms ($r = 0.62$).

We next tested whether the UFS stay had an immediate or on-going effect on nasal, ocular and pulmonary symptoms of grass pollen-allergic patients (Fig. 5). We observed a significant down-regulation of ocular, nasal and pulmonary symptoms ($p < 0.001$ in all cases) on the UFS (Fig. 5A–C). Both nasal and pulmonary symptoms continued to stay low also during the post-UFS interval (Fig. 5B, C). Only ocular symptoms increased again during the post-UFS interval, again showing an immediate effect of pollen, but never exceeded the half of the values of the pre-UFS levels (Fig. 5A).

3.6. Factorial model of symptoms, pollen and meteorological factors

When checking the interaction effects of several meteorological factors with airborne grass pollen concentrations on the symptom scores of allergic patients, we found that only relative humidity consistently and significantly correlated with pollen levels and with symptoms (Fig. 6). More specifically, in all three kinds of symptoms, higher pollen concentrations alone correlated with higher symptom scores. However, when relative humidity increased beyond approximately 60%, the respective threshold of pollen responsible for triggering symptoms decreased, viz. symptoms occurred at similar magnitude but with only half the pollen abundance. Particularly for pulmonary symptoms (Fig. 6C), when relative humidity exceeded around 70%, the positive correlation of pollen and symptom score ceased (as relative humidity exhibited a confounding effect on pollen abundance), but at the same time relative humidity alone caused increased pulmonary symptoms even without the co-effect of pollen.

When similar effects were investigated in the non-atopic cohort, it was found that nasal symptoms were positively correlated with relative humidity alone and regardless of pollen abundance ($p = 0.034$, $r = 0.35$; data not shown here).

3.7. Circadian patterns of ocular, nasal and pulmonary symptoms

At the 8-hourly timescale, ocular and nasal symptoms were significantly higher in the afternoon ($p = 0.012$, ocular symptoms; $p = 0.014$, nasal symptoms; *t*-tests for dependent samples), but this was true only for the pre-UFS stay of allergic patients; the same diurnal pattern was found also in airborne pollen concentration (see also Fig. 2B for comparisons). A delay effect of pollen was found on allergic symptoms of up to 16 h ($p < 0.01$ for both symptom forms, $r = 0.33$–0.38 for ocular symptoms, $r = 0.29$–0.36 for nasal symptoms; data not shown). This delay effect of several hours was also evident by correlating the

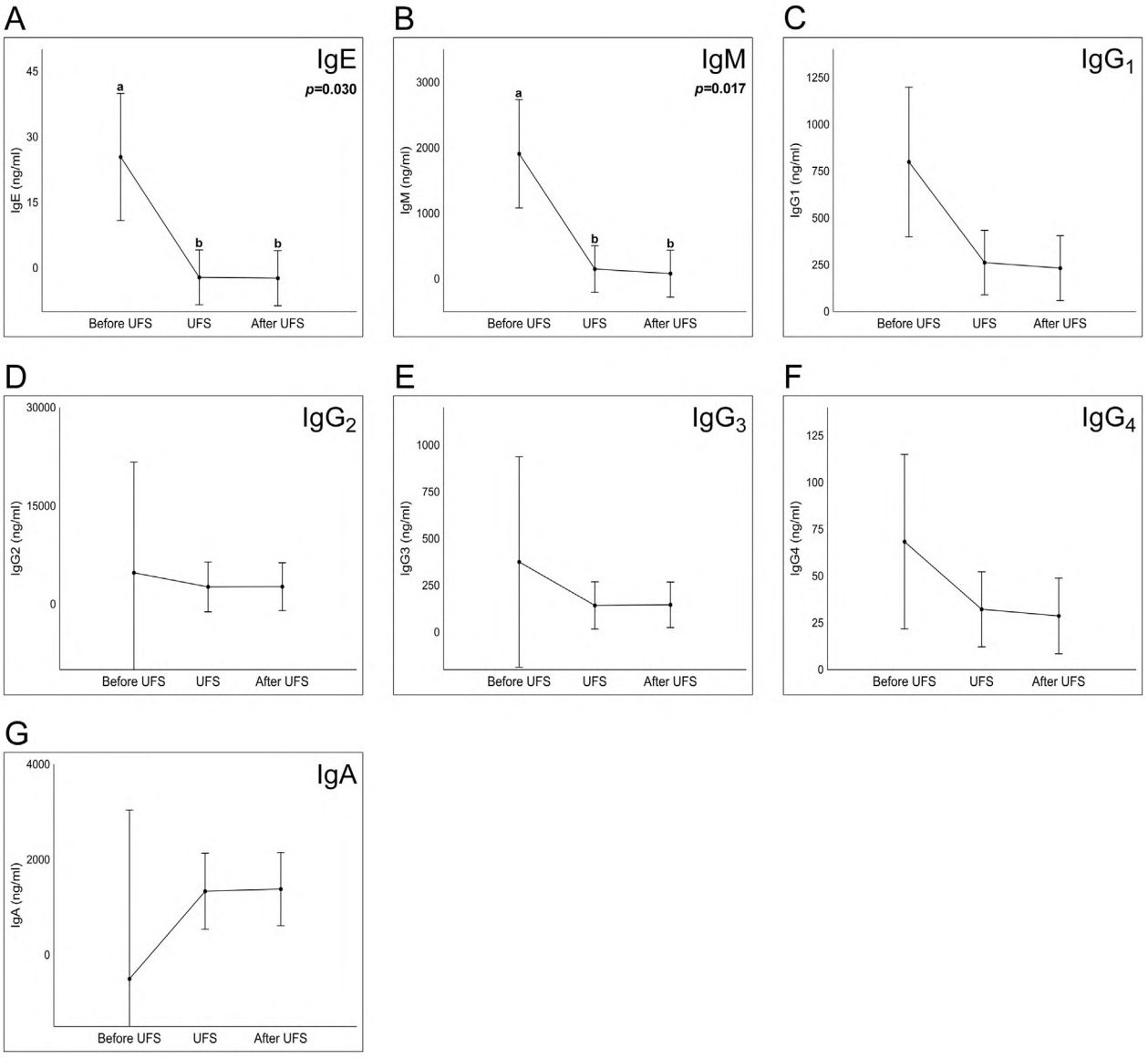

Fig. 3. Differences in levels of total immunoglobulins among study sites and dependent on pollen abundance. A–G: Comparisons of levels of total nasal immunoglobulins (Ig) of different isotypes among sites (categorical predictor) and pollen concentration (covariate) (ANCOVA). a, b: Significant differences after Bonferroni correction (a > b). Significance level *p* is indicated for significant cases.

symptom scores against the number of hours spent outdoors per day, including exercising hours: the most significant correlation, and positive, was again seen in the afternoon symptoms, both ocular and nasal ($r = 0.53$ and $r = 0.59$, respectively; data not shown).

4. Discussion

In this study, we compared spatiotemporal patterns of airborne grass pollen during peak flowering season between two fundamentally different geoclimatic environments, urban Augsburg and alpine Zugspitze, and then correlated these patterns with pollen allergic symptoms and immune mediators in a patient cohort. Our original hypothesis was that by lowering pollen exposure we would reduce symptom severity. Our hypothesis was indeed supported by our findings, similarly to previous results (e.g. Bastl et al., 2014; Berger et al., 2013; Karatzas et al., 2014; Osborne et al., 2017; Voukantsis et al., 2015).

We additionally found that this relationship was valid for all symptom forms (ocular, nasal pulmonary). It was true for different bioclimatic regions (urban vs. alpine), with both a direct relationship plus a delayed effect, with a repeated circadian pollen-symptom interaction pattern relying on the pollen abundance pattern but with a lag effect, and, finally, relative humidity decreasing the pollen threshold value beyond which symptoms are triggered. To our knowledge, such relationships for different forms of symptoms, lag effects with pollen and particularly meteorological parameters and, especially, at finer timescales have never been investigated.

Pollen abundance was lower on the alpine environment, as has been documented in other studies before (i.e. Charalampopoulos et al., 2013). However, on higher elevations there is also a higher mixing of the atmosphere and hence we still observed pollen, even while snowing, probably as an indication of long-distance transport. Such incidents have been recently reported for several different pollen taxa, including grass pollen, and for up to 2 km above ground level (Damialis et al., 2017). For this reason, pollen exposure is not probable to be eliminated completely even in the most 'unhospitable' environment, which also means that the potential allergy risk cannot be eliminated either. Moreover, outdoor pollen abundance was consistently higher than indoors up to a 6-fold magnitude, which also makes pollen allergies more relevant for outdoor exposure.

Allergic symptoms were found to correlate most significantly with airborne pollen concentrations of the same day, suggesting that

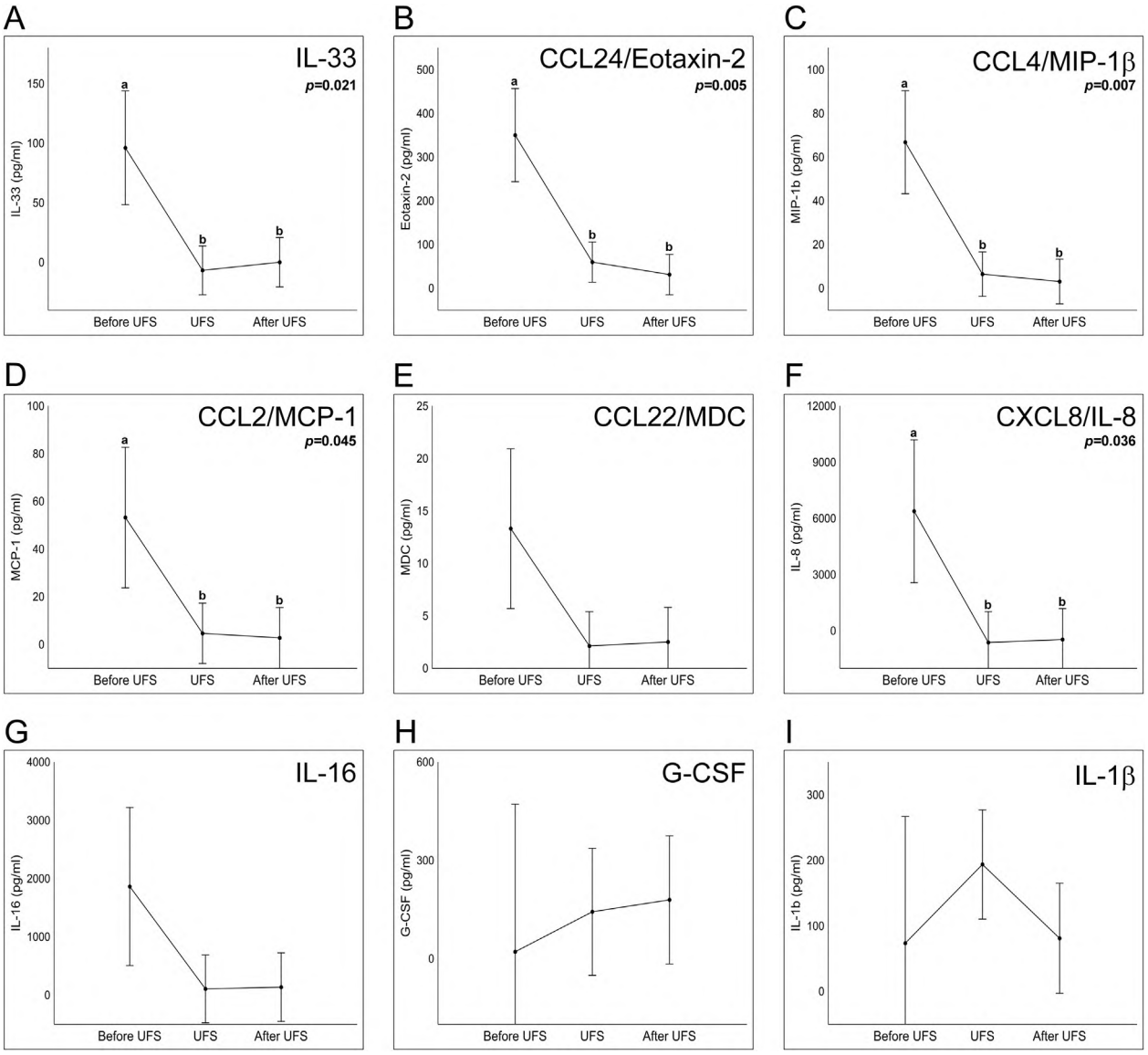

Fig. 4. Differences in levels of cytokines and chemokines among study sites and dependent on pollen abundance. A–I: Comparisons of levels of nasal proinflammatory cytokines and chemokines among sites (categorical predictor) and pollen concentration (covariate) (ANCOVA). a, b: significant differences after Bonferroni correction (a > b). Significance level *p* is indicated for significant cases.

immediate type immune responses, such as IgE-mediated activation of mast cells and eosinophils, were important contributors to the symptom load in our cohort (Janeway et al., 2001). Our time series analysis additionally revealed the ability to significantly reduce symptoms after low pollen exposure, and keep them mild for up to two weeks, mainly for nasal or pulmonary symptoms. However, ocular symptoms

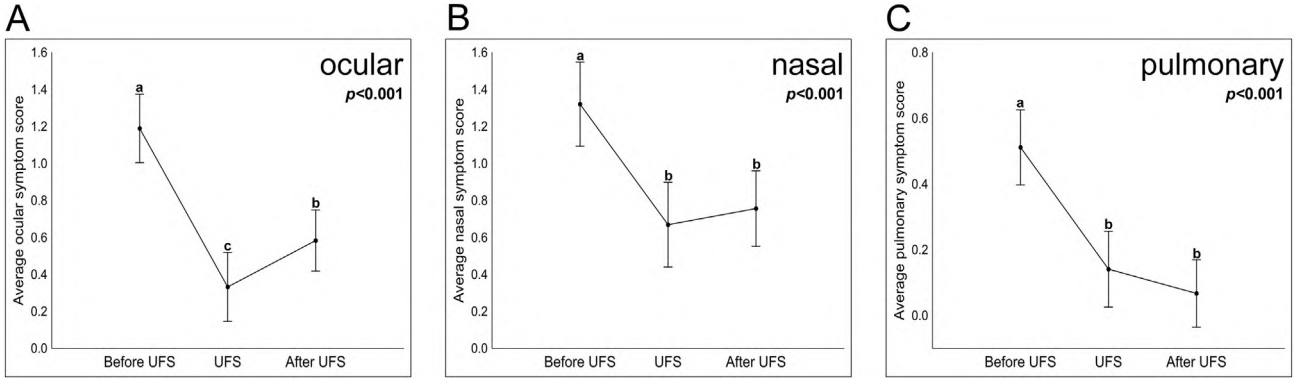

Fig. 5. Differences in symptom scores among study sites and dependent on pollen abundance. A–C: Comparisons of ocular, nasal and pulmonary symptom scores among sites (categorical predictor) and pollen concentration (covariate) (ANCOVA). a, b, c: significant differences after Bonferroni correction (a: the highest, c: the lowest). Significance level *p* is indicated.

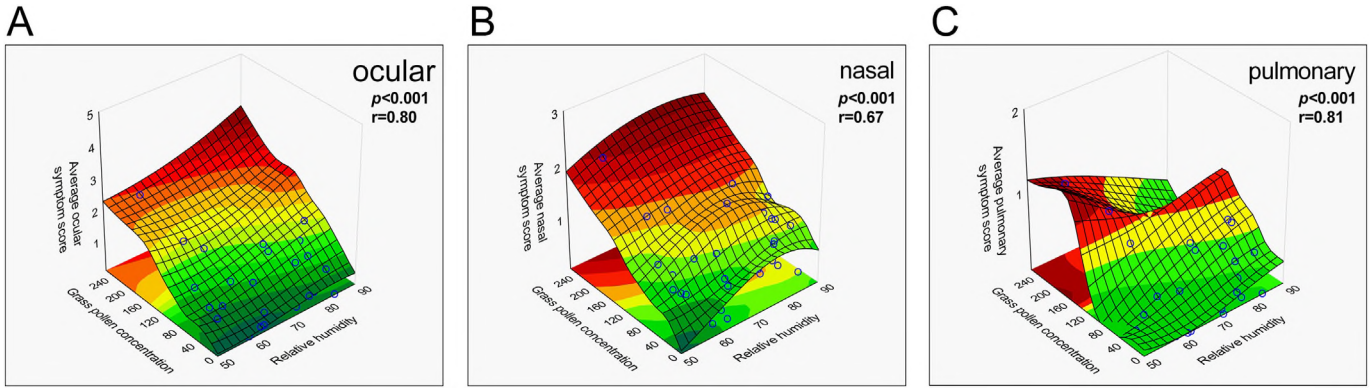

Fig. 6. Factorial models of symptoms, pollen concentrations and relative humidity. A: General Linear Models (factorial regression) of averaged symptom scores (A: ocular, B: nasal, C: pulmonary) (*z*-axis) against airborne grass pollen concentration (*y*-axis) and relative humidity (*x*-axis). Significance level *p* and Pearson correlation coefficient *r* are also given. The surface was fitted after the Least Square Difference method (stiffness = 0.2).

(Fig. 5A) and combination of symptoms (viz. total symptom score, Fig. 1) displayed a more immediate type response to increasing again pollen exposure.

The sustained reduction in symptoms is most likely explained by low pollen exposure during the first ten days of the UFS stay. Pollen counts as well as symptoms increased simultaneously after 10 June, even though still on the 'low exposure' UFS, as a result of the weather improving after a snowfall. It has to be considered that prolonged exposure with elevated pollen levels could have caused the patients´ symptoms to rise again to baseline levels, even on UFS. In this case, the beneficial effect would have eventually been lost. This means that even low-exposure environments can potentially be unsafe because of isolated or extreme events. In fact, climatic variations can cause high atmospheric pollen occurrence even in high alpine locations, as we indeed observed for UFS within the last 3 days of the patients´ stay. To assess the true contribution of climatic co-factors to the effect of mere allergen withdrawal, further studies should be carried out under natural exposure conditions, comparing symptoms in the same cohort between successive stays in different climatic regions, including a high-elevation, low humidity site. High altitude therapy regimes have been successfully applied for the treatment of chronic inflammatory diseases of the skin and airways (e.g. Bersuch et al., 2017; Fieten et al., 2018; Jung et al., 2012). The effect of high-altitude climate therapy on asthma was recently assessed in a systematic meta-analysis (Vinnikov et al., 2016), showing overall beneficial effects of high-altitude treatment mainly in adults, which did not differ between altitudes of 1560 m and >2000 m above sea level.

A unique feature of our current study design is the ability to monitor kinetics of symptoms and immune responses under an 'on-off-on' allergen exposure regime in the same patients. Consistent with a sustained reduction in symptoms, total nasal IgE and IgM levels decreased during the UFS stay and remained low, whereas total IgA levels tended to increase. IgA is found in large quantities in nasal fluid and is presumed to be crucial for immune exclusion at mucosal surfaces (Corthésy, 2013; Fujimoto et al., 2009). Nasal allergen-specific IgA_2 production has been linked to successful allergen-specific immunotherapy against grass pollen, suggesting a protective role in pollen allergy (Pilette et al., 2007). Nasal Igs are mainly directed against commensal or pathogenic microbes (Fujimoto et al., 2012). During nasal allergen exposure, however, specific Ig levels can increase dramatically. Since our study started during the main grass pollen season, it is likely that a large proportion of the total IgE measured in our allergic patients´ nasal samples was directed against pollen. This would explain the reduction following allergen withdrawal. The decrease in IgM likely reflects a generally reduced de novo maturation of B cell clones in local lymph nodes and nasopharynx-associated lymphoid tissues following lower pollen exposure (Brandtzaeg, 2011; Tamura et al., 1998).

Notably, levels of nasal IL-33, Eotaxin-2, MIP-1β, MCP-1 and IL-8 were reduced during the UFS stay and remained so throughout the rest of the study. This suggests sustainable effects of allergen withdrawal on the activation of type 2 innate lymphoid cells (ILC2) (Maggi et al., 2017) as well as on chemotaxis of eosinophils and neutrophils (Benson et al., 2006; Bocheńska-Marciniak et al., 2003; Erger and Casale, 1995), dendritic cell precursors (Robays et al., 2007) and T- and NK cells (Maghazachi et al., 1994). To our knowledge, this is the first study showing such profound changes in local immunoglobulin, cytokine and chemokine patterns under changing natural allergen exposure conditions. More extended studies designed in a similar way have the power to reveal novel kinetic features of the local immune response to natural aeroallergen exposure. They can also be designed to identify biomarkers in monitoring success of allergen-specific immunotherapy. The fact that nasal secretions are a completely non-traumatic, promising biomonitoring method could be of clinical relevance especially for the field of pediatric allergy.

When examining for co-factors that could explain more efficiently the cause-effect relationship between symptom severity and pollen abundance, we found that relative air humidity seems to lower the threshold concentration at which pollen cause symptoms. It was observed that relative humidity higher than 60% triggered symptoms with only half the amount of pollen normally needed, and this was particularly intense for pulmonary symptoms. Surprisingly, even non-atopic individuals exhibited nasal symptoms, irrespective of pollen, but dependent on increasing relative humidity. Further investigations would clarify this issue. Overall, below the approximate threshold of 70%, relative humidity alone does not play a dramatic role apart from favouring airborne pollen dispersion (Šaulienė and Veriankaitė, 2012). Such relationships with relative humidity were in the past found with respiratory symptoms in schoolteachers in classrooms, with either very low (<30%) or elevated relative humidity (>50%) correlating with increases in allergic and asthma-like symptoms (Angelon-Gaetz et al., 2016). On the other hand, an epidemiological study from Busan, Korea (Jo et al., 2017), from three years of data of hospital admissions due to respiratory diseases and meteorological factors showed that hospitalisations increased with rising air temperatures, rising PM_{10} concentrations and decreasing relative humidity. Under outdoor allergen exposure, it is likely that relative humidity acts in combination with site-specific meteorological and/or environmental confounders, as well as with climatic adaptation characteristics specific for the studied population. Control of respiratory allergic symptoms has been linked to an optimum in air humidity, with both dampness and extremely dry air as aggravating co-factors (Manuyakorn et al., 2015). Overall, it is well known that the definition of such thresholds comprises a highly demanding and complicated task, with those values varying among sites, countries, geoclimatic regions, among years and per pollen type

(de Weger et al., 2013). Integrating additional co-variables, like meteorological factors, could assist in resolving this issue. Indeed, our findings highlighted that the interaction of pollen and relative humidity was universal even when comparing as diverse ecosystems as alpine vs. urban. To our knowledge, the relationship between airborne pollen concentrations, relative humidity and respiratory symptoms has never been systematically analysed. The results of our pilot study point out the need for further studies, preferably controlled aerosol exposure chamber experiments testing the effect of pollen exposure under different air humidity regimes, mainly with respect to allergic asthma.

5. Conclusion

Low airborne pollen exposure efficiently reduces the symptoms and immune responses of pollen allergic patients. This decrease is persistent for nasal or pulmonary symptoms and immune responses and is retained for up to two weeks even if pollen exposure increases again into moderate levels. However, we need to emphasise that in extreme environments people are at the same time set under environmental stress and, thus, become symptomatic more easily, even under occasional or lower pollen exposure during only short intervals. Our results suggest that medical recommendations on allergy management need to take into account the whole variety of environmental factors influencing the allergic disease rather than only immune responses or symptom registries.

Acknowledgements

The study was partly financed by the Bavarian Ministry of the Environment and Consumer Protection (ZKL01Abt7-65149).

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