Ragweed Pollen Concentration Predict Seasonal Oculo-rhinitis and Asthma Severity in Subjects Allergic to Ragweed.

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Research Article

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Abstract

Objectives: 1. To investigate the correlation between ragweed pollen concentration and conjunctival, nasal and asthma symptoms severity in patients allergic to ragweed using ambient pollen exposure in the Milan area during the 2014 ragweed season; 2. to calculate the pollen / symptom thresholds and 3. to assess the effectiveness of ragweed Allergen Immuno Therapy (AIT).

Patients: 66 subjects allergic to Amb a 1 enrolled in the study and were divided into two cohorts: AIT treated (24) and non-AIT treated (42).

Measurements: Pollen counts and daily symptom/medication patient diaries. Autoregressive Distributed Lag Models were used to develop predictive models of daily symptoms and to evaluate the short-term effects of temporal variations in pollen concentration on the onset of symptoms.

Results: We found significant correlations between ragweed pollen load and the intensity of symptoms, for all three symptom categories respectively, both in non-AIT treated (τ= 0.341, 0.352, 0.721 and ρ = 0.48, 0.432, 0.881, p-value < 0.001) and in AIT treated patients (O= 0.46, 0.610, 0.66 and ρ = 0.692, 0.805, 0.824; p-value < 0.001). In both cohorts, we observed a positive correlation between the number of symptoms reported and drug use. Mean symptom levels were significantly greater in non-AIT treated than in AIT treated patients ($p < 0.001$) for all symptom categories. Pollen concentration thresholds for three symptom severity levels were calculated.

Conclusions: Ragweed pollen concentration is predictive of symptom severity in ragweed (Amb a 1) allergy patients. AIT treated patients had significantly reduced mean symptom levels compared to non-AIT patients.

Introduction

Ambrosia artemisifolia (Common Ragweed) is an invasive plant whose highly allergenic pollen causes seasonal respiratory allergy in people in many countries around the world (1-4). In particular, the North-West metropolitan area bordering the city of Milan (Italy) is one of the most infested areas with ragweed in Europe (5-7). Currently, the Agenzia di Tutela della Salute (ATS) is the regional agency responsible for healthcare management in the Metropolitan Area of Milan. Inthe North-West area it comprises two districts: ASST Ovest Milanese and ASST Rhodense (Fig. 1). Since the 90s, the ragweed plant has spread enormously in this area, becoming the leading cause of oculorhinitis and asthma in the summer-fall seasons. For example, an epidemiological survey in the municipality of Magenta, found that the prevalence of cases of ragweed oculorhinitis increased in the general population from 9.2–14.00% in the period from 1996 to 2005, and in the same period the prevalence of ragweed asthma increased by more than 40% (8). Furthermore, in 2012 a survey by the ATS Health Agency, involving the Allergy Units in the two districts, found that 54% of the patients visited for the first time for oculorhinitis, and 38% of those hospitalized for asthma, were allergic to ragweed (9). Given the high prevalence of ragweed allergy sufferers in this area, it is important to achieve an adequate control of oculorhinitis and asthma symptoms, as well as to limit the social and psychological consequences of the allergy during ragweed season. In fact, ragweed allergy in this area is a major limiting factor for people in schools andat work, affecting their learning ability. Consequently, since the 90s, a series of primary prevention measures have been carried out by the Province of Milan and the Lombardy Region in collaboration with the local Health Agency (today ATS). More recently, these preventive measures have become part of the COST SMARTER action (Sustainable management of *Ambrosia artemisiifolia* in Europe) (10). In practice, the following measures have been carried out in recent years: i) control of the territory, e.g. through aerobiological monitoring, surveillance and monitoring of the infested area, ii) urban planning, iii) epidemiological studies, and iv) studies on the effectiveness of various methodologies to limit the spread of ragweed, i.e.: mowing the weeds before flowering, covering the land, plowing the soil, harrowing discs and chemical control (11-15). Furthermore, since 2013 this area has been infested with a beetle (*Ophraella communa*) (16-20), which feeds preferably on ragweed weeds, causing them to dry out and die. As a result of these preventive measures and the beetle infestation, in the years following 2013 there has been a reduction in both the number of ragweed plants and the levels of airborne ragweed pollen (21).

However, reducing the concentration of pollen in an area, does not make the conclusion certain that there will be a reduction in the allergy symptoms in the residents of that area. More specifically, concerning the north-western area of the Metropolitan Area of Milan, to our knowledge,there are no published studies on the correlation between the concentration of airborne ragweed pollen and the severity of allergy symptoms. Indeed, in other countries only a few studies have been carried out on this topic and their results are conflicting: some have found a positive correlation between the concentration of ragweed pollen and the severity of both oculorhinitis and asthma (22-25), while others have not found any correlations (26-28), and two studies have even found an inverse correlation between ragweed pollen concentration and symptom severity (29, 30).

Given the ambiguity of these findings, we planned a cohort study investigating the correlation between the concentration of ragweed pollen and the severity of seasonal oculorhinitis and asthma in subjects allergic to ragweed. The second aim of our study was to determine theconcentrations of ragweed pollen to be considered as thresholds for the onset and worsening of symptoms. Finally, our third aim was to evaluate the effectiveness of AIT in reducing the severity of ragweed allergy symptoms.

Methods

Study design

The primary goal of this study was to investigate the association between exposure to airborne ragweed pollen and daily ocular, nasal and asthmatic symptoms in two sub-cohorts of patients throughout one whole ragweed season. We followed individuals sensitized to ragweed and suffering from seasonal ragweed oculo-rhinitis with/or without asthma, from July 16 to September 15, 2014. Study subjects weredivided in two cohorts: one consisted of individuals who had never received a ragweed AIT, hence “non-AIT”,while subjects in the other cohort had been treated with ragweed AIT, hence “AIT treated”. Patients were treated with AIT prior to, and independent of, their enrollment in the study. These patients received AIT treatment either in the same year as the study or in the three years immediately preceding the study.

Originally, we actually envisioned a different study design. Ragweed and mugwort coexist in the study area, and since mugwort and ragweed blooms are partly overlapping, at firstwe also wanted to investigate the influence of mugwort exposure on allergy symptoms attributed to ragweed. However, since the number of patients with dual sensitization (i.e. ragweed and mugwort) was very small (n=25), the original study design was modified to include only subjects sensitized to ragweed.

From July 16, 2014 to September 15, 2014, all patients filled in a daily Clinical Diary of Symptoms and Drugs (CDSD). During the same period, pollen counts were measured from three pollen traps located in the study area (see the 'Pollen concentration' section for exact locations). The daily averages of ragweed and mugwort pollen concentration obtained from the three pollen traps were used for statistical calculations against the daily mean of the symptom/drug scores for each of the two sub-cohorts.

Setting

Patients were recruited and followed in the areas of the ASST Ovest Milanese and ASST Rhodense Districts, both of which are under the milanese health protection agency ATS (Fig. 1). Both districts are located in the North West area of the Metropolitan Area of Milan, covering an area of approximately 827 sq. km (geographical coordinates: to the East: Lat 8 ° 39' 59" E, to the North: Long 45° 35' 54" E, to the West: Lat 9 °11' 47" E, to the South: long 45°16' 46" N (with the exclusion of the Municipality of Milan)). Patients within the two districts were enrolled in the Allergy Units of the following hospitals: Legnano Hospital (Long 45° 35' 44" N, Lat 8° 55' 23" E), Abbiategrasso Hospital (Long 45° 23' 40" N, Lat 8° 54' 49" E), Garbagnate Milanese Hospital, Pneumology and Pediatric Allergy Units (Long 45° 34' 53" N, Lat l9° 05'14" E), Cesano Boscone Ambrosiana Clinic (Long 45°26' 57" N, Lat 9° 05' 33" E). Each one of these five Allergy Units was located in proximity of one of the three pollen traps (i.e. min-max distance: 0.2 -19.6 km) (Fig. 1).

Participants

Participants in the study were a random sample of citizens residing in the designated area, with a confirmed diagnosis of ragweed seasonal rhino-conjunctivitis (with or without asthma), some of which were treated with AIT. 66 subjects (male: 32, female: 34) were enrolled in the study. The Eligibility Criteria to participate in the study were: 1) subjects were sensitized to ragweed pollen, demonstrated by a positive SPT with commercial ragweed extracts (Lofarma SpA, Milan; ALK-Abellò SpA, Milan) and IgEs for the total ragweed extract and for rAmb a 1 (pectate lysase) (Immuno -CAP Thermo Fisher Scientific Inc., Monza, Italy). In case of discordant test results, ragweed sensitization was confirmed based on positive IgEs for rAmb a 1. 2) Subjects had an established and documented history of clinical manifestations of ragweed allergy (i.e. symptoms of oculo-rhinitis, associated or not with asthma), coinciding with the ragweed flowering period, in the years immediately preceding enrollment. 3) Subjects had given their informed consent. 4) Subjects were able to adhere to the study protocol, and 5) subjects would remain in their residence throughout the observation period.

All eligiblestudy subjects also performed SPT with 12 other inhalant allergens, namely pollens of grass, birch, hazel, alder, mugwort, *D.pteronyssinus* and *D. farinae*, Aspergillus, Cladosporium, Alternaria, cat and dog dandruff and, moreover, IgEs for the total mugwort allergen, rArt v 1 (defensin-like protein linked to the polyproline-rich region) and rArt v 3 (NS LTP type 1).

At the end of the recruitment period, from February 01, 2014 to July 15, 2014, 71 subjects were assessed to the Eligibility Criteria. One subject was excluded because he did not perform the IgE test and four because the IgEs for rAmb a 1 were negative. The remaining 66 patients were confirmed eligible and were included in the study. The mean age was 36.5 years (range: 8 - 69 years). None of the subjects were pregnant, nor suffering from chronic diseases. All subjects resided in the study area, and in the period from February 01, 2014 to July 15, 2014, they had an allergic visit for respiratory symptoms in one of the five Allergy Units participating in the study. Moreover, 42 subjects had never been treated with AIT for ragweed, while 24 subjects had previously received ragweed AIT.Thus, patients were divided into two sub-cohorts: non-AIT and AIT treated. All recruited subjects (100%) completed the follow-up and were subjected to statistical analysis. The characteristics of the study base at enrollment are shown in Table 1.

Table 1
Characteristics of the study base at enrollment

	non-AIT **n= 42**	**AIT treated** **n= 24**
Male	23 (55%)	9 (37,5%)
Female	19 (45%)	15 (62.5%)
Mean age at enrolment (range)	34.47 (8-69)	39.58 (11-62)
Allergy / Sensitization		
rAmb a 1	42/42 (100%)	24/ 24 (100%)
rArt v 1	14/ 42 (33%)	7/24 (29%)
rArt v 3	4/42 (9.5%)	3/24 (12.5%)
Asthma	17/42 (40%)	11/24 (46%)

Pollen concentration data

Ragweed and mugwort's pollenwas sampled daily from three Hirst-type pollen traps located respectively in Legnano (Lat 45° 35' 44" N, Long 8° 55' 23" E), Magenta (Lat 45° 28' 16" N, Long 8° 53' 33" E) and Rho (Lat 45° 32' 51" N, Long 9° 02' 42" E). The Hirst volumetric trap continuously draws 10 liters of air per minute onto an adhesive coated tape. Particles in the air stick to the tape, which moves at 2mm per hour to provide a time sample. The pollen collected from the traps was identified and quantified by a specialized technician and then correlated to the average air volume over 24 hours. The reference standard adopted for the sampling and counting of pollen was the UNI 11108 of 2004, valid at the time of the study.

The following definitions were adopted from the Quality Control Working Group of the European Society of Aerobiology (EAS) and the International Association of Aerobiology (IAA) (31). Pollen count: result of the analysis on the slide; this quantity is not comparable and needs to be converted into concentration. Pollen grain: male gametophyte of the seed plant. Pollen concentration: expressed as pollen grains/m^3, i.e. the number of pollen grains dispersed in the air per unit of air volume. Main Pollen Season (MPS): the length of time that pollen is present in the atmosphere in significant concentrations in a place. The MPS in our study was based on the average of the three monitoring stations using the Nillson & Persson criterion (32): "the period from when the sum of the average daily pollen concentrations reaches 5% of the total sum up to at the time when the sum reaches 95%; i.e. the main pollen season with 90% of the entire quantity of pollen".

Clinical Diary of Symptoms and Drugs (CDSD)

All study participants were asked to complete a CDSD during the follow up period. Patients were asked to record their daily symptoms and medication use every evening from July 16th to September 15th, 2014. Each symptom was rated on a 4-point scale: 0: no symptoms; 1: mild symptoms; 2: moderate symptoms; 3: severe symptoms. These ratings were used to score each one of the following 9 distinct symptoms: Nasal: 1. nasal congestion/nasal difficulty of breathing, 2. runny nose, 3. itchy nose, 4. itchy throat/ears; Ocular: 5. itching and/or burning in the eyes, 6. tearing and/or wet eyes; Bronchial: 7. cough, 8. breathing difficulty while moving, 9. breathing difficulty at rest. Therefore, the total symptom score ranged from 0 to 24, while the nasal score ranged from 0 to 9, the ocular from 0 to 6 and the bronchial from 0 to 9. Furthermore, patients were also asked to record every single unit of drug taken, i.e. number of tablets/day of oral antihistamines, number of spray/day of inhaled topical nasal corticosteroids or topical bronchial corticosteroids or topical bronchial corticosteroids + Long Acting Bronchodilator Agents (LABA) or + Short Acting Bronchodilator Agents (SABA), and number of drops/day of antihistamine eye drops.

Patient follow up during the exposure period

All patients enrolled in the study went to their reference Allergy Unit immediately before the exposure period began. During the visit, their clinical history was collected, a medical examination was performed and patients gave their informed consent for participation in the study. All patients were also provided with a CDSD, which was returned compiled for all dates during the second visit after September 15, 2014.

Comparability of evaluation methods.

Patients in both cohorts, non-AIT and AIT treated, were recruited for the study in the same way, underwent the same diagnostic tests, received the same CDSD, were visited in the follow up on the same dates, and received the same Informative Consent.

Bias

The known overlap in the initial time period of ragweed MPS with mugwort MPS was a possible cause of bias with respect to the symptom score attributed to ragweed, given that the symptoms possibly caused by exposure to mugwort pollen are indistinguishable from those caused by ragweed pollen in subjects sensitive to both pollen species. However, the concentration of mugwort pollen detected during the study period was very low (see below) and therefore, unlikely to have influenced in any significant way the respiratory symptoms of those patients also allergic to mugwort.

Other possible causes of bias on the symptom/drug score may have been: climatic variables, air pollution, as well as any intercurrent respiratory infectious diseases (which were not considered in orderto simplify the analyses).

Ethics

The study protocol complies with the Declaration of Helsinki and all its subsequent amendments of Tokyo 1975, Venice, 1983, Hong Kong, 1989, as well as with the current regulations for good clinical practice (GCP). The protocol also complies with all national and community regulations applicable to observational studies and all ethical and deontological principles that inspire the medical profession. The study was approved by the Ethics Committee of Milan Area C (No 80_122013).

Quantitative variables.

Only ragweed pollen concentration was considered as a feature in the regression models,since the correlation between symptom/drug score and mugwort pollen concentration was overall low and not significant.

Statistical analyses

Spearman's rank ρ correlation coefficient (33) and Kendall's rank τ correlation coefficient (34) nonparametric statistical tests were used to examine the correlation between daily ragweed pollen concentrations and symptoms' intensity, measured as the total daily number of symptoms observed in study patients. We adopted a non-parametric statistical approach as the target variables were not normally distributed.

The non-parametric Kruskal-Wallis test (35) and the Wilcoxon rank sum test (36) were used to compare mean symptom level scores between the two patient cohorts: non-AIT and AIT treated.

Time series analysis was used to analyze the influence of the daily pollen concentrations cycle on the onset of allergic symptoms. It is reasonable to assume that the total number of symptoms currently observed depends on the daily pollen concentrations, as well as on the previous day's (lag=1) symptom values. For this reason, we applied a first-order autoregressive distributed lag (ARDL) model to explore these short-run relationships (37).

Let x_t(t=1, ...,T) be the daily pollen concentrations and $y_t$the total number of symptoms observed in the study patients. The ARDL model is defined by

$$y_t = \alpha + \varphi_1 y_{t-1} + \theta_0 x_t + \epsilon_t, \quad \text{(t=1, ...,T)} \quad (1)$$

where $\boldsymbol{\varepsilon}_t$ is a white noise process, independent of x_t, y_t and y_{t-1}, so that the model (1) can be estimated using ordinary least squares (OLS). The regression coefficients can be interpreted as measures of the influence of the feature on the target variable.

We also considered a first-order autoregressive distributed lag model similar to the model formulated in (1) with the addition of a lagged x_{t-1} as a further explanatory variable,

$$y_t = \alpha + \varphi_1 y_{t-1} + \theta_0 x_t + \theta_1 x_{t-1} + \epsilon_t, \quad \text{(t=1, ...,T)} \quad (2)$$

Furthermore, the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) were used for model selection. Models with the lowest AIC and BIC values were considered to be the ‘best’. Finally, since the pollen concentration data showed high variance, we used a square root transformation to stabilize the variance.

All statistical analyses were conducted using R 4.0.2 (38).

Results

Pollen counts.

Figures 2-4 show the average daily ragweed and mugwort pollen concentration values from the three pollen traps during the follow-up period.

Ragweed

The ragweed MPS began on August 18, 2014 and ended on September 18, 2014, while the peak pollen period (PPP) extended from August 25, 2014 to September 10, 2014, with a cut of 25 pollen grains/m^3. During the MPS, the maximum (C max) and the daily mean (C mean) concentrations of ragweed pollen reached by the individual trap stations were as follows: Legnano-trap (C max = 75 pollen grains/m^3, C mean = 23 pollen grains/m^3), Magenta-trap (C max = 469 pollen grains/m^3, C mean = 52 pollen grains/m^3) and Rho-trap (C max = 145 pollen grains/m^3, C mean = 27 pollen grains/m^3). The maximum concentration from the averages of the three stations was 211 pollen grains/m^3(note: this value does not correspond to the mathematical average of the C max of the individual stations). Since people movedaround the studied area, in the analyses we decided to use the daily average concentration of the three stations.

Mugwort

The mugwort MPS for 2014 was calculated from the average of the three monitoring stations and ran from August 10, 2014 to October 04, 2014. No real mugwort PPP was observed, since the average C max of the three stations did not exceed the value of 10 pollen grains/m^3. During the mugwort MPS, we observed a higher concentration of pollen only during two periods: (1) from August 11, 2014 to August 21, 2014 (Fig. 3)and (2) from September 17, 2014 to October 04, 2014 (which was outside the follow-up time and thus not included in the figure). However, these mugwort peaks were very low and belonged to two different species(*Artemisia vulgaris*in the first peak and *Artemisia verlotorum* in the second).

The mugwort C max recorded by the individual trap stations were as follows: Legnano-trap 10 pollen grains/m^3, Magenta-trap 16 pollen grains/m^3 and Rho-trap 15 pollen grains/m^3. The C max obtained from the averages of the three stations was 10 pollen grains/m^3(note: this value does not correspond to the mathematical average of the C max of the individual stations).

Statistical analyses

Table 2 reports the descriptive statistics relating to the average number of daily symptoms per person per symptom category, i.e conjunctivitis, rhinitis and asthma.

Table 2

Descriptive statistics: mean, median, 25% quantile (Q1), 75% quantile (Q3), and standard deviation (sd), related to the average number of daily symptoms per person for each category of symptoms.

Symptoms	Group	mean	median	Q1	Q3	sd
conjunctivitis	non-AIT	0.873	0.738	0.595	1.143	0.347
	AIT treated	0.343	0.333	0.208	0.458	0.189
rhinitis	non-AIT	1.201	1.048	0.714	1.768	0.544
	AIT treated	0.673	0.625	0.458	0.865	0.264
asthma	non-AIT	0.385	0.357	0.286	0.500	0.117
	AIT treated	0.157	0.083	0.042	0.208	0.149

Table 3 shows the results of the non-parametric correlation tests for the two patient cohorts: non-AIT and AIT treated. A moderate significant correlation between ragweed pollen load and symptoms intensity was found in the non-AIT cohort ($\tau = (0.341,0.352,0.721)$and $\rho = (0.48,0.432,0.881)$, p-value<0.001), while a strong significant correlation was found in the AIT treated cohort (τ=(0.46, 0.610, 0.66)and $\rho = (0.692,0.805,0.824)$, p-value<0.001)for all three categories of symptoms respectively. Moreover, in non-AIT patients, we observed a strong positive significant correlation (τ= (0.656, 0.711, 0.648) and ρ= (0.821, 0.878, 0.818) with a p-value <0.001) between the number of symptoms and drug consumption (see Table 4). In AIT treated patients such correlation was more moderate but still significant (τ= (0.495, 0.600, 0.399) and $\rho =$ (0.628, 0.777, 0.516) with a p-value<0.001).

Table 3

Results of the correlation tests between the number of symptoms and pollen concentration: values of Sperman's (ρ) and Kendall's (τ) rank correlation coefficient and their significance.

Pollen	Symptoms	Group	Sperman		Kendall	
			ρ	*p*-value	τ	*p*-value
ragweed	conjunctivitis	non-AIT	0.480	<0.001	0.341	<0.001
		AIT treated	0.692	<0.001	0.469	<0.001
	rhinitis	non-AIT	0.432	<0.001	0.352	<0.001
		AIT treated	0.805	<0.001	0.610	<0.001
	asthma	non-AIT	0.881	<0.001	0.721	<0.001
		AIT treated	0.824	<0.001	0.661	<0.001
	Symptoms		**Sperman**		**Kendall**	
			ρ	*p*-value	τ	*p*-value
mugwort	conjunctivitis	non-AIT	0.293	0.021	0.229	0.018
		AIT treated	0.224	0.080	0.167	0.084
	rhinitis	non-AIT	-0.003	0.982	-0.003	0.974
		AIT treated	0.074	0.568	0.035	0.712
	asthma	non-AIT	0.222	0.083	0.132	0.168
		AIT treated	0.083	0.523	0.042	0.668

Table 4
Results of the correlation tests the number of symptoms and drug consumption: values of Sperman's (ρ) and Kendall's (τ) rank correlation coefficient and their significance.

Symptoms	**Group**	**Sperman**		**Kendall**	
		ρ	*p*-value	τ	*p*-value
conjunctivitis	non-AIT	0.821	<0.001	0.656	<0.001
	AIT treated	0.628	<0.001	0.495	<0.001
rhinitis	non-AIT	0.878	<0.001	0.711	<0.001
	AIT treated	0.777	<0.001	0.600	<0.001
asthma	non-AIT	0.818	<0.001	0.648	<0.001
	AIT treated	0.516	<0.001	0.399	<0.001

Hereafter, we consider only the ragweed pollen concentration as a feature in the regression models, because the correlation between symptoms' intensity and mugwort pollen concentration was low and overall not significant.

Figure 2 and Figure 3 show the total number of symptoms observed in patients for all three symptom categories, and the mean daily ragweed and mugwort pollen concentrations (pollen grains/m^3) averaged over all three traps. In the non-AIT cohort, the total number of daily symptoms increased through time from mid July to mid September: symptoms increased a few days after the rise in airborne ragweed pollen concentrations, continuing to increaseup to their peak value and further, due to the known late response to the allergen. We used the Kruskal-Wallis and the Wilcoxon rank sum teststo test the hypothesis that mean symptom levels in non-AIT patients were greater than those in the AIT treated group. We found significant differences between daily symptoms in the two groups at a 95% confidence level or higher (Table 5).

Table 5
Kruskal-Wallis test and Wilcoxon rank sum test results for comparing non-AIT and AIT treated mean symptom levels (alternative = "greater").

	Kruskal-Wallis test			**Wilcoxon rank sum test**	
Symptoms	Chi-squared	df	p-value	W	p-value
conjunctivitis	28.397	17	0.041	3840.0	<0.001
rhinitis	45.513	20	0.001	3737.5	<0.001
asthma	29.445	12	0.003	3735.5	<0.001

Figure 4 shows drugs use in the two patient cohorts, AIT treated and non-AIT treated, and the daily ragweed and mugwort pollen counts, averaged over thethree stations.

Table 6 presents OLS estimates of the ARLD models (1), considering as covariate the square root of the ragweed pollen daily concentration (m^{-3} day^{-1}). We also estimated model (2), but we selected model (1) according to the AIC and BIC values. We assumed that the target variable y_t, i.e. the total number of symptoms at a given day t, may be explained in terms of current x_t i.e. the square root of the ragweed pollen concentration, and past symptoms values, y_{t-1}.

Table 6
Estimation of the Autoregressive Distributed Lag (ARDL) model (1) in the two cohorts of patients for conjunctivitis, rhinitis, and asthma-like symptoms. (Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1)

Symptoms	***Group***				
conjunctivitis	*non-AIT*				
			Estimate	**Std. Error**	**p-value**
		intercept	6.098	2.036	0.004 **
		y_{t-1}	0.745	0.069	1.49e-19 ***
		x_t	1.221	0.33	0.0005 ***
		Adjusted R-squared: 0.865; AIC:383.418; BIC: 391.861			
	AIT treated				
			Estimate	**Std. Error**	**p-value**
		intercept	2.444	0.923	0.01 *
		y_{t-1}	0.593	0.1040	4.23e-7 ***
		x_t	0.307	0.155	0.052.
		Adjusted R-squared: 0.444; AIC:327.397; BIC: 335.841			
rhinitis	*non-AIT*				
			Estimate	**Std. Error**	**p-value**
		intercept	7.068	2.443	0.005 **
		y_{t-1}	0.784	0.069	2.67e-16 ***
		x_t	1.540	0.523	0.005 **
		Adjusted R-squared: 0.918; AIC: 407.305; BIC: 415.748			
	AIT treated				
			Estimate	**Std. Error**	**p-value**
		intercept	3.219	1.267	0.014 *
		y_{t-1}	0.765	0.089	6.07e-12 ***
		x_t	0.269	0.185	0.1517
		Adjusted R-squared: 0.694; AIC:330.904; BIC: 339.347			
asthma	*non-AIT*				
			Estimate	**Std. Error**	**p-value**
		intercept	3.242	1.06	0.003 **
		y_{t-1}	0.743	0.081	8.34e-13 ***
		x_t	0.348	0.132	0.011 *
		Adjusted R-squared: 0.844; AIC: 258.518; BIC: 266.961			
	AIT treated				
			Estimate	**Std. Error**	**p-value**
		intercept	0.220	0.341	0.521
		y_{t-1}	0.75	0.076	6.01e-14 ***
		x_t	0.239	0.091	0.011 *
		Adjusted R-squared:0.768; AIC:245.455; BIC: 253.899			

Figure 5 shows the bi-dimensional exposure-response relationship estimated by ARDL models. Each panel can be read using two different perspectives: it represents the increase in number of total symptoms in each t + k future day following a single exposure equal to ℓ (ℓ = 1, 5, 10, 20) ragweed pollen per cubic meter of air at day t = 0 (forward interpretation), or otherwise the contributions of each t − k past day, with ragweed pollen per cubic meter of air equal to ℓ (ℓ = 1, 5, 10, 20), to the increase in number of total symptoms at day t (backward interpretation).

In order to define the airborne ragweed pollen threshold levels for the protection of human health, we considered the quantiles of order (0.25, .5, .75) for the total number of symptoms (without distinguishing them in categories) per person in non-AIT patients in the period from August 01, 2014 (the first date in which the average number of ragweed pollen was greater than 1) until September 02, 2014 (the ragweed pollen peak). The quantiles defined four classes based onthe total number of symptoms: low, medium-low, medium-high, and high. The threshold levels corresponding to each class were obtained by computing the averaged ragweed and pollen concentrations (pollen grains/m^3) for the corresponding days, i.e. L1= 0.963, L2= 4.233, and L3 =14.444.

Data on other sensitizations were not used in the elaboration of the results as they were not significant for the purpose of our study.

Discussion

Key results

The first objective of our study was to test the hypothesis that the severity of symptoms of ragweed hay fever is directly related to the concentration of ragweed pollen in the air. The results of our study support this hypothesis. First, we found a strong significant correlation in AIT treated patients, and a moderate significant correlation in non-AIT patients, between ragweed pollen load and symptoms' intensity for all three symptom categories (i.e. conjunctival, nasal and bronchial). Moreover, in the non-AIT cohort, we observed a strong positive correlation between the number of symptoms and drug consumption. In the AIT treatedcohort this correlation was more moderate but still statistically significant. Second, the OSL estimates of the ARLD models (1) demonstrated that the daily ragweed pollen concentration is predictive of the severity of each of the symptom categories considered.

The second objective of our study was to establish threshold values of ragweed pollen concentrations for different levels of symptom severity. We were able to establishthree different average ragweed pollen concentration thresholds, each corresponding to a different symptom intensity level, i.e. L1 = 0.963, L2 = 4.233, and L3 =14.444.

Our third objective was totest the hypothesis that exposure to equal concentrations of ragweed pollen, during ragweed MPS, would result in less severe ocular, nasal and bronchial symptoms in AIT treated patients compared to non-AIT. We found that the mean symptom levels for the three symptoms categories were significantly greater, over the entire observation period, in non-AIT patients than in AIT treated. The mean daily symptom scores and the mean daily drug consumption scores in the AIT treatedgroup were lower, each day, compared tothenon-AIT group. Furthermore, the ARDL model (1) estimate indicated that the increase in pollen level has a greater contribution on the increase in symptoms in non-AIT patients compared to AIT treated.

Limitations

The main implicit limitation to the first objective's results is that the symptom score attributed to ragweed pollen may have been influenced by the coexistence of mugwort pollen. This may have occurred both in some subjects sensitized to mugwort, as well as in non-mugwort sensitized subjects, due to a cross-reaction between ragweed and mugwort allergens (39). In fact, of the 66 ragweed sensitized subjects participating in the study, 21 had specific IgE for Art v 1, and 7 for Art v 3. In 2014,the mugwort MPS partially overlapped with ragweed MPS during the study's symptom/drug-monitoring period (i.e. from July 16, 2014 to September 15, 2014). However, during the entire study period, mugwort pollen concentrations were extremely lower than ragweed concentrations, and did not show any peaks. Furthermore, mean symptom scoreswere never significantly positively correlated to mugwort pollen concentration values, suggesting that symptom scores did not depend on mugwort pollen concentration. Also the extremely low C max value of mugwort pollen during the 2014 mugwort MPS, supports our conclusion that the mugwort pollen concentration did not influence the symptom scores of the study participants.

Other study limitations could arise from other factors that can affect the severity of respiratory symptoms. For example, we did not consider air pollution, which is particularly intense in the study area, due to the high industrial and commercial development. The same applies to possible intercurrent respiratory infections that, as far as we know, did not occur, but for which there was no specific provision for reporting them in the CDSD.

The calculated threshold values of ragweed pollen concentration related to symptom severity may also have limitations. In fact, it is known that in the air we find not only allergens contained in the pollen grains, but also free allergens in the form of bioaerosols. For example, studies found that when the concentration of ragweed pollen is 200 pollen grains/m^3, contemporarily 2.5 mg/m^3 of free ragweed allergens are also present (40-43). Consequently, a symptom threshold based on pollen counts alone cannot give a precise quantification of true exposure to all airborne allergens. Furthermore, atmospheric and environmental factors may alsosometimes influence the results. Indeed, the amount of free allergen associated with pollen grains depends on a number of variables, including air humidity (which can influence the release of the allergen-containing starch from pollen grains), rain, wind and the trauma that pollen suffers in the environment, such as that caused by intense car traffic (43). Moreover, there is evidence that ragweed pollen collected along high-traffic roads has a higher allergenicity than pollen collected in vegetated areas (44).

Concerning the third study objective,possible limits and biasesin the results could come from not having considered some of the AIT treatment variables, i.e. the definition of the allergenic composition of the extracts used, the maximum and cumulative dose administered, the dose of the main allergens administered, the route of administration and treatment duration.

Interpretation

The results of this study indicate that there is a correlation between ragweed pollen concentration and the severity of ocular, nasal, and bronchial symptom scores in ragweed allergy sufferers. The data are valid for the experimental conditions of our study, and we exclude a confounding effect due to a coexisting mugwort allergy. Based on the available variables, the correlation data and OLS estimates of the ARLD models (1) show a strong relationship between ragweed pollen concentration and symptom severity, supportingour main hypothesis. We observed a greater correlation between the number of symptoms and ragweed pollen concentrations in AIT treated subjects compared to non AIT, with regard to conjunctivitis and rhinitis. This result could be due to the fact that conjunctivitis and rhinitis are treated with OCD, since they are considered less severe symptoms than asthma. This explanation is also supported by the finding of a strong correlation between the number of symptoms and drug consumption, which is greater in non-AIT than in AIT treated subjects. It is likely that AIT treated subjects tend to take fewer drugs because they feel protected by the immunotherapy. This phenomenon is not observed in asthma, where there is no difference in the correlations between the number of symptoms and pollen concentration in AIT and non-AIT treated subjects.

Future studies should take into consideration all of the AIT variables (as mentioned above). However, under similar experimental conditions, we do not expect that these additional variables would critically affect the results.

In the literature, the majority of studies investigating the association between pollen load and symptom severity, are not specifically aimed at ragweed pollen, or have not specifically selected populations of subjects allergic to ragweed (26-30). A few studies that investigated exposure to ragweed pollen in populations of ragweed allergy patients, do support our results. Della Valle et al. (24) found a strong association between daily asthma symptoms and drug scores, and ragweed pollen concentrations in ragweed-sensitized children. They studied a total cohort of 430 children (4-12 years old), sensitized to different pollens, from which ragweed-allergic children had been enucleated and separately studied. Caillaud et al. (25) also selected only ragweed allergy patients (32 adults) and found a linear relationship between rhinitis, conjunctivitis and asthma symptom scores and ragweed pollen counts. Likewise, Newhouse et al. (22) found a significant correlation between ragweed pollen and rhinitis, and asthma symptoms in 24 ragweed allergy patients (aged 9 to 64) in Tulsa. The importance of including only patients selectively allergic to the specific pollen under examin the study is stressed by Brito et al. (45), which found a significant correlation between concentration of olive pollen with symptoms of rhinitis and asthma in 20 patients mono sensitized to olive pollen in Ciudad Real.

Other factors that are important to consider when conducting similar studies are: selecting a sufficient number of patients sensitized to ragweed, recruiting only subjects with demonstrated sensitization, placing the pollen traps close the subjects' residences, and recording both symptom and drug consumption scores.

The results of our study probably depend on having selected a random sample of patients havingboth an established sensitization to ragweed (to rAmb a 1) and confirmed seasonal respiratory symptoms from ragweed allergy, residing in a relatively small area (a few km2), highly infested with ragweed, and living very close to one of the three pollen traps. Moreover,inthe analyses we used the daily average of the three pollen-traps counts, which is likely to be a very representative variable of the quantity of pollen to which patientswere exposed daily in that area.

Furthermore, even though we recognize its limits, calculating a threshold for symptom pollen levels is an important toolfor setting ragweed-allergy preventative measures. Unfortunately, comparisons with the results of previous studies are limited. In fact, while a number studies have determined symptom thresholds for grass and birch pollen, similar studies for ragweed pollen are practically non existent. An olderstudy(24), where almost all patients with a ragweed allergic rhinitis were symptomatic, proposed a range between 10 to 50 pollen grains/m^3 as threshold level. Another study (46) established the ragweed pollen concentration threshold for the onset of hay fever symptoms at 1-3 pollen grains/m^3.. Lastly, a study (47) on environmental triggers of asthma in children,foundthat a threshold of 6-9 weed (not ragweed) pollen grain/m^3 could trigger asthma symptoms. Unfortunately, the data provided by these studies cannot be used for comparison with ours. Dataon ragweed pollen symptom thresholds can be useful for individual prevention only if they are publishedtogether with data on pollen concentrations. Studiesthat measure pollen-symptom thresholds along with aerobiological information do exist, but validated criteria on how to provide this information to the public are still lacking (48-51). Nonetheless, it can be very useful to know the symptomatic thresholds; since they can be used as a standard for all preventative measures carried out in an area, with the aim of reducing the ragweed plant's expansion.

Finally, the results of our study also support the hypothesis that subjects treated with AIT have less severe symptoms upon exposure to the same concentration of ragweed pollen, compared to subjects not treated with AIT. Even though there are limitations to these results due to some unmeasured AIT variables, such as the characteristics of the ragweed extracts used, the duration and methods of administration. Nonetheless, our general aim was to highlight the fact that AIT can modify the severity of symptoms from exposure to ragweed pollen in treated subjects. The model we used can be adopted to evaluate a specific ragweed extract, or a particular route of administration, or duration of AIT. Our experimental model satisfies the requirements cited by the EAACI Position Paper (52). Here again, comparisons with previously published studies on the "real world" effectiveness of AIT are limited. In fact, we could not find any published "real world" comparative studies of patients treated with AIT pollen, versus untreated patients, similar to our study model. We believe that our results are unprecedented in this respect. Although not directly comparable to our study, some "real world" studies compared patients treated with AIT for different seasonal allergens, for more than one year of treatment, and with untreated control subjects. In these studies, AIT effectiveness was confirmed based only on one significant difference in symptom/drug score in AIT treated subjects versus controls (53-56).

Generalizability

Our data were collected in a population of subjects allergic to ragweed (all residing in a well-defined area, highly infested with ragweed plants) and with a high prevalence of seasonal conjunctival, nasal and bronchial symptoms due to sensitization to ragweed. Therefore, our results can be generalized in situations with similar contexts. We strongly believe that our findingsthat ragweed hay fever symptoms are positively correlated with ragweed pollen concentration, are generalizable to many areas of the world where there are high concentrations of airborne ragweed pollen. Furthermore, our results have significant implications for the metropolitan area of Milan, as they confirm the validity of the efforts made to contain the expansion of the ragweed plant in the territory.

The threshold levels of pollen exposure related to the severity of symptoms established in our study are probably not generalizable. Nevertheless, they are in line with those reported in an olderstudy (47). At the moment, given the absolute dearth of confirmatory studies, our data remain unique and can be used as an indicative reference for planning various territorial interventions.

Finally, our findingsshow that AIT is a treatment able to reduce the symptoms caused by exposure to the allergen but, in the short term, it is not able to completely eliminate these symptoms. We believe this conclusion confirms the opinion ofmany experienced allergistsover AIT.

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Figures

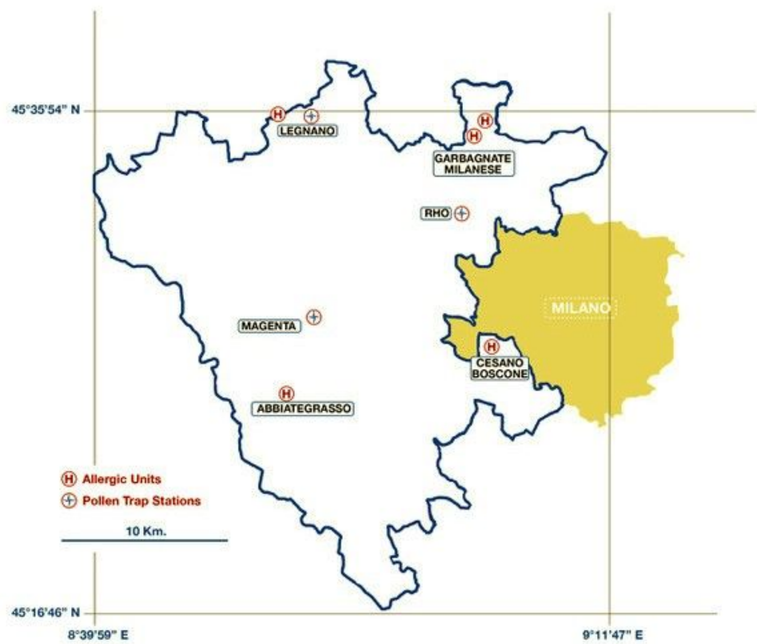

Figure 1

Map of the ASST Ovest Milanese and ASST Rhodense Districts, included in the Agency For Health Protection of the Metropolitan Area of Milan (ATS); the 'blue +'symbols represent the three Hirst volumetric traps, and the 'red H'shows the location of the five allergy clinics (note: in Legnano there is both an Hirst volumetric trap and an allergy clinic)

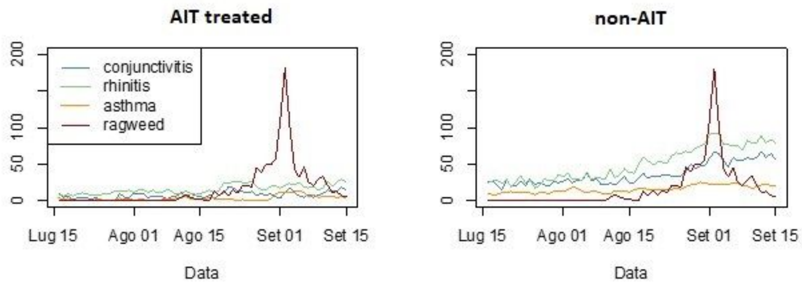

Figure 2

Time series of observed total number of symptoms, for the three categories, in the two patient cohorts: AIT treated (left) and non-AIT (right), and ragweed mean daily pollen concentrations. Concentrations are given as daily mean values and are expressed in gram per cubic meter of air (pollen grains/m^3)

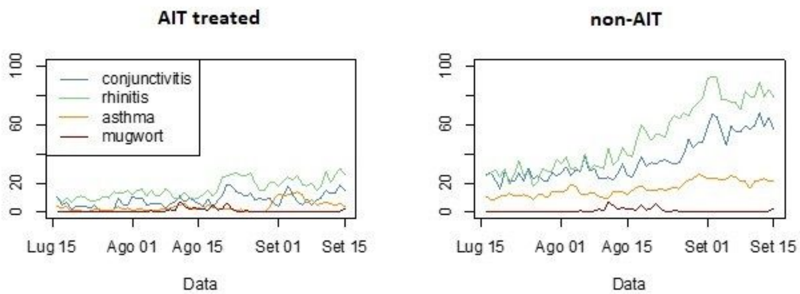

Figure 3

Time series of observed total number of symptoms for the three categories in the two groups of patients: AIT treated (left) and non-AIT (right), and mugwort mean daily pollen concentrations (pollen grains/m^3)

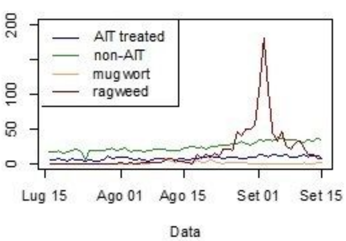

Figure 4

Time series of observed drugs use in the two cohorts of patients: AIT treated and non-AIT, and ragweed and mugwort pollen concentrations (pollen grains/m^3)

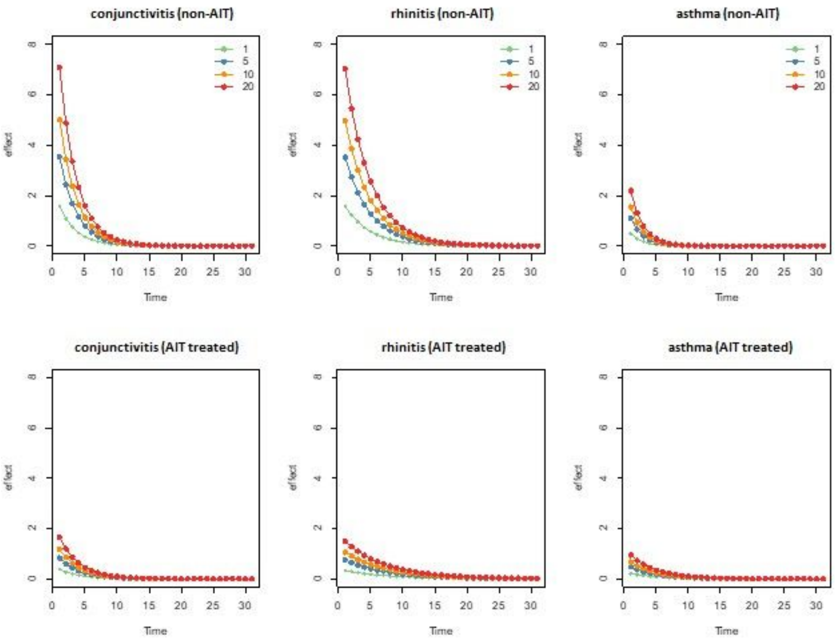

Figure 5

Effects of different increasing levels ℓ (ℓ =1, 5, 10, 20) of ragweed pollen concentrations (pollen grains/m^3) on the total symptoms score in “AIT treated” and “non-AIT” patients for conjunctivitis, rhinitis, and asthma-like symptoms