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The influence of sensitisation to pollens and moulds on seasonal variations in asthma attacks

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Abstract

No large study has described seasonal variation in asthma attacks in population based asthmatics in whom sensitisation to allergen has been measured.

2,637 young adults with asthma living in 15 countries reported months in which they usually had attacks of asthma and had skin prick tests performed. Differences in seasonal patterns by sensitisation status were assessed using generalised estimating equations.

Most young adults with asthma reported periods of the year when their asthma attacks were more common (range 47% in Sweden to 86% in Spain). Seasonal variation in asthma was not modified by sensitisation to house dust mite or cat. Asthmatics sensitised to grass, birch and *Alternaria* had different seasonal patterns to those not sensitised to each allergen, with some geographical variation. In Southern Europe, those sensitised to grass were more likely to report attacks occurred in spring/summer than in winter (ORMarch/April=2.60; 95%CI 1.70-3.97; ORMay/June=4.43; 95%CI 2.34-8.39) and smaller later peaks were observed in Northern Europe (ORMay/June=1.25; 95%CI 0.60-2.64; ORJuly/August1.66; 95%CI 0.89-3.10). Asthmatics reporting hayfever but who were not sensitised to grass showed no seasonal variations.

Seasonal variation in asthma attacks in young adults is common and is different depending on sensitisation to outdoor, but not indoor, allergens.

KEYWORDS: asthma exacerbation, ECRHS, hay fever, pollen, seasonality.

Introduction

As far back as the 5th century BC Hippocrates noted seasonal variation in exacerbations of asthma [1]. Seasonal patterns have been described for asthma mortality, hospital admissions and emergency department visits [2-5], the variation being ascribed to seasonal variation in exposure to ambient allergens, respiratory infections and metereological changes [4,6]. In older adults, asthma mortality and hospital admissions are more common during the winter months (possibly explained by infections), and in the younger adult population (a 'more atopic' generation) are more common in periods of high ambient allergen load (grass in the spring and summer, mould in the late summer and autumn) [7].

However, to date, no large study has described seasonal variation in population-based asthmatics who also have information on IgE sensitisation. Small panel studies suggest asthma severity is worse in the pollen season in those sensitised to grass [8,9], and in winter in those sensitised to house dust mite [10]. Asthmatics presenting to Accident and Emergency in Spain in early summer (May-June) were more likely to be sensitised to grass than those presenting in other months [11]. Similarly, mild, near fatal and fatal asthma has been associated with sensitisation to *Alternaria* in months where *Alternaria* levels are high [12,13].

In this report we use information collected from participants in The European Community Respiratory Health Survey (ECRHS), a large multicentre international study of young to middle-aged adults living in differing climatic regions and in countries with marked variation in the prevalence of sensitization to environmental allergen [14]. The aim is to describe seasonal variation in asthma exacerbations in those who are and are not sensitised to aero-allergen, to quantify the risk and to assess whether seasonal patterns are consistent between countries.

Material and methods

The ECRHS I [15] recruited a random sample of at least 3,000 adults aged 20-44 years identified from a population based sampling frame in each participating centre. Each was sent a postal questionnaire and a random sample of responders plus a sample of those with symptoms suggestive of asthma (symptomatic sample) were invited for further tests. The postal survey, the clinical interview (at which skin prick tests were performed and blood samples taken) and the testing of samples for serum IgE were conducted between 1991-1993.

Overall 41 centres in 16 countries and 3 continents (Europe, North America, Australasia) followed the full protocol. Of the 24,115 participants (random AND symptomatic sample), 3,353 (13.9%) reported they 'ever had asthma' and 3,151 (94%) provided a complete response to the question 'Which months of the year do you usually have attacks of asthma?' (with a 'no' or 'yes' response to each of six bimonthly periods January/February, March/April, May/June, July/August, September/October, November/December). Overall 2,709 (86%) had skin prick tests. In one centre (Iceland) the number of asthmatics with positive SPTs was insufficient for statistical analysis leaving 2,637 asthmatics with information on seasonality and SPTs (2,023 with serum specific IgE and total IgE measures) (Figure 1 on line repository).

Skin prick tests (SPT) to Timothy grass, *Dermatophagoides Pteronyssinus* (house dust mite), cat, *Cladosporium herbarium*, *Alternaria alternata*, birch and common ragweed were performed using Phazets, lancets precoated with standardized lyophilized allergen extracts (Pharmacia Diagnostics AB, Uppsala, Sweden). SPTs were considered positive if the mean wheal diameter (MWD) was greater than 0 mm [16]. Serum samples were tested for specific IgE (Timothy grass, house dust mite, cat, *Cladosporium* plus birch in Northern Europe, Parietaria in Southern Europe and ragweed in Australia and US) using the Pharmacia CAP System. Sensitization was considered present if serum IgE was >0.35 kU/L. Due to

regulatory and ethical constraints SPT to Timothy grass was not performed in Germany. Ethical permission to conduct the study was granted by local ethics committees within each participating centre.

Statistical Analysis

Each participant was considered to have taken part in six surveys one asking if they experienced asthma attacks in January/February, another in which they were asked whether they experienced attacks in March/April and so on. A marginal logistic regression model for binary outcomes, based on generalised estimating equations (GEE), was used to generate the risk of reporting a particular two month period was associated with attacks of asthma (with January/February as the reference category). This method, appropriate for dealing with longitudinal and other correlated binary response data, generates robust estimators regardless of the specification of the covariance matrix, and as autocorrelation is included in the covariance, coefficients can be interpreted as usual. The bi-monthly periods were shifted 6 months forward for Australian and New Zealand centres to allow seasonal comparison across the two hemispheres.

To examine whether these patterns were different in those who were and were not sensitised, an interaction term (positive skin prick test to specific allergen no/yes X the six bi-monthly periods) was included giving a total of 5 interaction terms for each model. Where there was evidence of effect modification by sensitisation analyses were repeated stratified by sensitisation status. Throughout, adjustments for age, gender and sensitisation to the allergens not under assessment (four of house dust mite, grass, cat, *Alternaria*, birch) were included.

Analyses were conducted within country, but as there were insufficient data within each country to identify patterns clearly, results from each country were combined using meta-analytical techniques. Risk estimates for each country within each of the three continents

were combined in a random effects meta-analysis [17] and heterogeneity tested using the Q statistic and I^2. As there was evidence of heterogeneity in Europe, European countries were further divided into Central/North Europe and South Europe (defined by +/- 50° latitude). As this geopolitical method of division Europe may not fully capture pollen exposures, we also interrogated pollen maps, (available at http://www.pollenwarndienst.at) to divide countries into those with a) Poaceae season starting before and after May/June and b) countries where peak birch season levels were in excess of 70.0 grains/m^3 (see on-line Table 1-2 for more information).

In sensitivity analyses, the above was repeated defining a positive SPT positivity by MWD >=3mm, with sensitisation based on serum IgE measures (using both >0.35kU/L and >0.70 kU/L cut offs), with the sample restricted to asthmatics who reported symptoms (wheezing, chest tightness, attack of shortness of breath coming on during the day at rest, waking with shortness of breath) or the use of asthma medication in the last 12 months (n=2,423, 92%) and with inclusion of adjustment for month of interview. Effect modification of seasonality of asthma attacks by high total IgE (>100kU/L) and reported 'hayfever or nasal allergies' was also assessed.

All analyses were undertaken in Stata IC 10.1 (Stata software version 10.1; Stata Corp., College Station, Texas, USA).

Results

Table 1 shows the number (range 43 in Ireland to 387 in Canada), and age and gender distributions, of participants with self reported asthma in each country. Of these 93% reported 'physician diagnosed asthma'. The proportion of asthmatics sensitised to each allergen varied between countries in a pattern broadly similar to that previously reported in the general population [14] (Table 1). Allergic sensitisation to allergens associated with vegetation

(grass, birch), indoor allergens (cat, dust), and moulds (*Alternaria*) was prevalent in all countries. However the prevalence of sensitisation to *Cladosporium* was low in all countries except USA (n=12, 18%), Canada (n=57, 15%), Sweden (n= 51, 14%) and UK (n=46, 13%). Similarly the prevalence of sensitisation to ragweed was low in most countries except Canada (n=104, 27%), USA (n=11, 16%) and UK (n=16, 4%). The small number of participants precluded inclusion of USA in analyses of variation by these two allergens.

Table 2 shows the proportion of asthmatics who, when asked, reported their attack of asthma usually occurred at specific times of the year and the adjusted odds ratio of reported asthma attacks in each bi-monthly period. In most countries, most participants reported seasonal variation in their asthma (range 47% Sweden to 86% Spain; median 70% in France, Switzerland, New Zealand). In some countries there was no clear overall seasonal pattern (e.g.: France, Ireland, Germany and Norway), whereas in many countries (e.g.: Spain, Italy, Switzerland, Belgium, Sweden, USA and Australia) a significant increased risk of reported asthma attacks was seen in spring/summer months (March/April, May/June) compared to winter ones. In contrast, there was an overall decreased risk of asthma in the summer months in Netherlands, UK, Canada and New Zealand (i.e. asthmatics reported their attacks of asthma usually occurred in winter).

There was no evidence that sensitisation to house dust mite or cat modified the seasonal pattern of reported asthma attacks in any of the countries/continents (p for interaction of bimonthly period by sensitisation >0.05, I^2 for variation between countries <40%) (Figure 1a 1b). In the few centres with sufficient data there was some suggestion of effect modification by having a positive skin prick test to ragweed (Canada, UK) throughout the summer and early autumn ($p<0.05$ only in September/October) and by having a positive skin prick test to *Cladosporium* in July/August (UK, Sweden, Canada) but formal testing showed this to be below the conventional limits of statistical significance (Figure 1c 1d).

In contrast there was evidence that seasonal variation was modified by sensitisation to grass, birch and *Alternaria* (Figures 2-4 in online repository show meta-analysis of interaction coefficients). In Figures 2-4, the odds for reporting asthma attacks in each bimonthly period is shown stratified by sensitisation status. There was, however, considerable variation in Europe (for example I^2 for interaction term for grass in May/June and birch sensitisation in March/April within Europe 61% and 76% respectively). When Europe was considered as North and South or divided based on available average pollen levels over the last fifteen years (early/late Poaceae season or by peak birch pollen levels reached) some differences still remained.

In both South and North Europe (Figure 2a-b) those sensitised to grass were more likely to report asthma attacks usually occur in the spring/summer periods than in the winter period while those who were not sensitised were more likely to report attacks in the winter months. For those who were grass sensitised the higher risks were in early summer in Southern Europe (OR $_{\text{March/April}}$ 2.60 95% CI 3.17-3.97; OR $_{\text{May/June}}$4.43 95% CI 2.34-8.39), and by comparison, in Northern Europe the peak risks were relatively smaller, occurred later and showed more variation between the countries (OR $_{\text{July/August}}$ 1.66 95%CI 0.89-3.10; I^2 61%). Similar patterns were seen in Europe when countries were considered to have an early or late grass pollen season (Figure 5 in online repository). In North America (Figure 2c) the pattern was similar to that seen for Southern Europe (although all interaction terms $p>0.05$). In Australia and New Zealand (Figure 2d) the effect of grass sensitisation on asthma attacks was more complex, being different in the two countries, and superimposed on an overall decreased risk of attacks during the summer/autumn months. Formal testing for modification of the seasonal pattern by sensitisation to grass was significant ($p<0.05$) only in March/April. In all regions the seasonal pattern of asthma amongst those reporting 'hayfever or nasal allergies' (overall 77% of asthmatics also reported hayfever) was similar to that seen for grass

sensitisation (54% of those with hayfever also had sensitisation to grass) (Figure 5 in online repository). However, this pattern was only present in those sensitised to grass, and not seen in those who reported hayfever but who were not sensitised to grass (Figure 3).

In Europe, asthmatics who were sensitised to birch were more likely to report attacks occurred in the spring/summer than in the winter months. The seasonal pattern amongst those who were sensitised to birch was clearly seen in Northern Europe ($OR_{May/June}$ 2.94 95% CI 1.92-4.50; $OR_{July/August}$ 2.01 95%CI 1.38-2.94 Figure 4b), although there was some variation between countries (for example $OR_{May/June}$ 0.87 95%CI 0.55-1.37; I^2: 72%). The pattern was seen in countries where peak birch pollen levels exceeded 70.0 grains/m^3 (Figure 6 in online repository), but again there was some variation in the magnitude of effect between countries. . Compared to grass sensitisation this peak risk in this sensitised group occurred earlier in the year (Fig 3b 4b). Some seasonal variation was also seen in Southern Europe (where birch pollen levels are generally lower). There was no evidence that sensitisation to birch modified seasonal variation of asthma attacks in North America or Australasia (all interactions $p>0.05$ Figure 4c-d).

Asthmatics who were sensitised to *Alternaria* were at a greater risk of reporting asthma attacks in May/June and July/August in Southern Europe (Figure 5a) and in July/August in North Europe (Figure 5b). There was some evidence of similar seasonal patterns of reporting asthma attacks in North America (Figure 5c) (formal testing for interactions $p<0.05$ in July/August) and in Australasia (Figure 5d) the pattern was similar to that seen for grass sensitisation.

None of the above observations were substantially altered by changing the definition of a positive SPT (MWD≥3 mm), by using serum specific IgE to define sensitisation (using a cut off of 0.35kU/L or 0.70kU/L), by restricting the sample to those who had asthma symptoms in the last year or by including the month of interview (and skin prick test) as a potential

confounder. Furthermore, high levels of total IgE (>100 kU/ml) did not influence seasonal variations (data not shown).

Discussion

This large study of over 2,500 young adults with asthma shows that a substantial proportion report seasonal variation in their attacks of asthma, and that the pattern of the seasonal variation is dependent on whether they are sensitised to pollens and moulds, but not indoor allergens. Asthmatics sensitised to grass, birch and *Alternaria* show very different seasonal patterns to those not sensitised to these allergens. This is most clearly seen for sensitisation to grass, where individuals who are sensitised report more attacks in the summer months (most likely related to allergen exposure) and those who are not sensitised report more attacks in the winter months (most likely due to the effects of respiratory infections [6]). Although these patterns are widely recognised by clinicians, to date no epidemiological study of this scale has described these patterns, or derived risk estimates associated with sensitisation status in representative samples of the asthmatic population.

Beyond the clear relevance for clinical practice, our results provide important baseline information for risk assessment in relation to climate change and may assist in the interpretation of health effects of outdoor air pollution on asthma.

We would expect the seasonal pattern in those who are sensitised to pollens and mould to mirror the patterns of exposure. We do not have complete information on pollen levels in all of our participating centres for the period of data collection (1991-1992). Two groups (The European Academy of Allergy and Clinical Immunology and the International Association of Aerobiology) collated all available pollen data from Europe (1974-1988) [18] which showed peak grass (Poaceae) pollen counts occurred later in Northern Europe than Southern Europe, and in some countries (eg: Northern Italy) were raised well into September/October. In

Scandinavian countries the birch pollen season began in May lasting at least till the end of August being earlier shorter and more intense in 'Southern' Europe. The 'average' pollen season over the last fifteen years is broadly similar to these patterns (http://www.pollenwarndienst.at) and more recent European work confirms these temporal and geographical variations in grass pollen [19,20], birch [21] and mould spore counts (*Alternaria*, *Cladosporium*) [22,23]. However, there is growing evidence that measurement of pollen allergenicity (a subject of research within multicentre initiatives such as HIALINE http://www.hialine.com), rather than pollen counts, may be the relevant exposure to explain allergen associated variations in asthma exacerbations [24].

Our report suggests that mortality and admissions are the 'tip of the iceberg' of seasonal variations in asthma. Most asthma treatment guidelines list exposure to seasonal allergens as a potential trigger to asthma attacks [25] and our data support that those with sensitisation to grass, birch, *Alternaria* (and possibly *Cladosporium*) may benefit from focussed clinical protocols [26] that increase inhaled treatment during the months of peak exposure to allergen. They may even benefit from desensitisation and immunotherapy (2.6% of asthmatics reported they 'had been vaccinated for allergy in the previous 12 months'). Assessment of sensitisation by skin prick tests and/or serum measures may not be routinely performed in all primary health care settings. However, the interpretation of such testing requires understanding of potential cross-reactivity and should be carefully considered alongside information on likely exposure. For example, some people may test positive to ragweed in the UK, but ragweed pollen is absent. Sensitisation probably arises from cross-reactivity with other members of the Compositae (Asteraceae) family (eg: mugwort). Even though our data suggest that people with positive skin tests to ragweed in UK and Canada have different seasonal patterns, particularly in late summer, compared to those without positive skin prick tests, (Figure 1d) this might be explained by residual confounding by mould exposure.

Similarly the pattern of variation that we have seen in those who do and do not have positive skin prick tests to birch in Southern Europe may in part be explained by cross-reactivity with other members of the Bet v 1-family, (eg hazel and hop-hornbeam) [27]. Asthmatics who also reported hayfever showed an increased risk of reporting exacerbations in the spring and summer months but this was limited to those who were grass sensitised. Although reporting hayfever identifies those at greater risk of spring/summer asthma exacerbations (figure 7 online) the addition of testing for grass sensitisation may be more useful to identify those at risk (Figure 3). Indoor allergens such as house dust mite and cat may have a role in determining asthma exacerbations and severity of disease but our data suggest that those sensitised to indoor allergens have the same seasonal variations as those who are not sensitised to these allergens.

Large time series and ecological studies of asthma mortality and admissions in relation to pollen/mould exposure [7,28-32] present effect estimates for the entire population and have not included differences in effect in those who are and are not sensitised (the proportion of which varies between countries). The effect of pollen on sensitised individuals may confound the respiratory effects of other outdoor pollutants, particularly ozone, (which peaks during the summer months). This could explain the inconsistency in the association of ozone with asthma reported in some time series studies [33,34]. Possible interactions between air pollutants and aero-allergens on asthma exacerbations have been investigated in few studies. Individual sensitisation to such aero-allergens might influence the observed effects and explain why such interactions are only seen in some studies [35].

Climate change may lead to more intense and extended pollen/mould seasons with higher absolute levels and increased allergenicity [36,37]. There may be more thunderstorms which are known to cause sudden peaks in grass and mould allergen levels and ‘epidemic asthma’ in those who are sensitised [11,38]. This report provides baseline information showing that

those who are sensitised are indeed a subgroup of the population who will be particularly at risk if these climactic changes occur.

The ideal study design to investigate the effects of pollen exposure on asthma exacerbations is a longitudinal or panel study of asthmatics who provide symptom and sensitisation information at regular intervals over a one year period, but only one large study of this type (on children) has been reported [39]. Although our study design is not optimal it is to our knowledge the only large international study of seasonal variations in population based asthmatics in whom atopic status has been assessed. Participants were identified from representative community based samples (not from hospital clinics) and even though the vast majority reported a physician diagnosis of asthma, only a third reported using inhaled steroids in the last year (reflecting they had relatively mild disease [40] and the under-treatment of asthma during the 1990s). We have relied on self reported seasonality collected at one point in time which has high face validity but may introduce random error (making it more difficult to identify clear patterns). Systematic error is unlikely as seasonal reporting was not influenced by month of interview or by participants knowing their IgE status (questionnaire was asked before skin prick testing).

Even though our report is based on observations made 20 years ago, similar patterns were still seen in adults who took part in ECRHS II (a subsample of participants included in this report who were followed up in 2000-2002, n=1,284, aged 27 to 54 at follow-up, 12 countries) who underwent repeat questionnaires and limited serum IgE testing (no SPTs were performed in ECRHS II) (Figure 8 in online repository).

We have described geographical variation in seasonality but because of the relatively small sample size in some centres have grouped information from centres to country level, and then to regional level. Our regions are thus defined by geopolitics rather than factors that others may consider more relevant (eg: pollen levels, climate, land usage).

In conclusion, we have shown that seasonal variation in asthma attacks in young adults is common and is strongly associated with allergic sensitisation. This is a timely reminder to clinicians to remain alert to seasonal allergens as a potential trigger to asthma attacks in their sensitised patients. Air pollution scientists should strive to include measures of allergen exposure and measures of individual (or at least population levels) of allergic sensitisation in their analyses. Climate change may increase pollen levels and their allergenicity and also extend pollen seasons, this will likely lead to increased exacerbations in those who are sensitised to these aero-allergens. Large panel studies to better understand and quantify the relationships between exacerbations, allergic sensitisation directly measured pollen counts and other factors are required.

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Table 1. Demographic characteristics and the proportion of asthmatics with positive skin prick test to each allergen, by country (ordered by latitude)

		N	Gender (Women)	Age	DUST MITE		TIMOTHY GRASS		CAT		BIRCH		ALTERNARIA	
Continent	**Country**		**%**	**Mean ± SD**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Southern														
Europe	**Spain**	190	53%	31.7 ± 7.3	82	43%	53	28%	32	17%	11	6%	9	5%
	Italy	88	52%	32.9 ± 6.9	27	31%	41	47%	20	23%	19	22%	12	14%
	France	273	51%	33.1 ± 7.6	146	53%	113	41%	83	30%	33	12%	41	15%
	Switzerland	122	51%	31.8 ± 6.7	37	30%	75	61%	37	30%	58	48%	11	9%
Northern Europe	**Germany**	63	51%	34.3 ± 7.4	23	37%	28*	44%	22	35%	23	37%	6	10%
	Belgium	64	50%	33.1 ± 7.0	56	88%	23	36%	21	33%	11	17%	7	11%

	Netherlands	51	49%	34.3 ± 7.9	34	67%	16	31%	9	18%	11	22%	2	4%
	Ireland	43	56%	32.1 ± 7.0	36	84%	12	28%	7	16%	1	2%	3	7%
	UK	366	57%	33.2 ± 7.0	217	59%	179	49%	131	36%	44	12%	84	23%
	Sweden	354	56%	32.7 ± 7.5	107	30%	165	47%	219	62%	167	47%	48	14%
	Norway	60	48%	33.1 ± 6.6	13	22%	18	30%	16	27%	17	28%	2	3%
North America	**USA**	67	49%	33.6 ± 6.8	36	54%	31	46%	31	46%	18	27%	23	34%
	Canada	387	61%	33.1 ± 7.0	215	56%	143	37%	175	45%	89	23%	85	22%
Australasia	**New Zealand**	303	57%	34.3 ± 6.9	205	68%	148	49%	67	22%	54	18%	38	13%
	Australia	205	53%	33.6 ± 6.8	150	73%	111	54%	73	36%	19	9%	39	19%
Total		2,637	55%	33.2 ± 7.1	1,384	53%	1,128	44%	943	36%	575	22%	410	16%

Centres: Spain (Barcelona, Galdakao, Albacete, Oviedo, Huelva) Italy (Pavia, Turin , Verona), France (Paris, Grenoble, Bordeaux, Montpellier), Switzerland (Basel), Germany (Hamburg, Erfurt), Belgium (Antwerp City, South Antwerp), Netherlands (Groningen, Geleen, Bergen-op-zoom), Ireland (Dublin), UK (Ipswich, Norwich, Cambridge, Caerphilly), Sweden (Uppsala, Umea, Goteburg), Norway (Bergen), USA (Portland), Canada (Winnepeg, Vancouver, Hamilton, Montreal, Halifax, Prince Edward Island) New Zealand (Wellington, Christchurch, Hawkes Bay), Australia (Melbourne) *Germany figure based on serum IgE measure

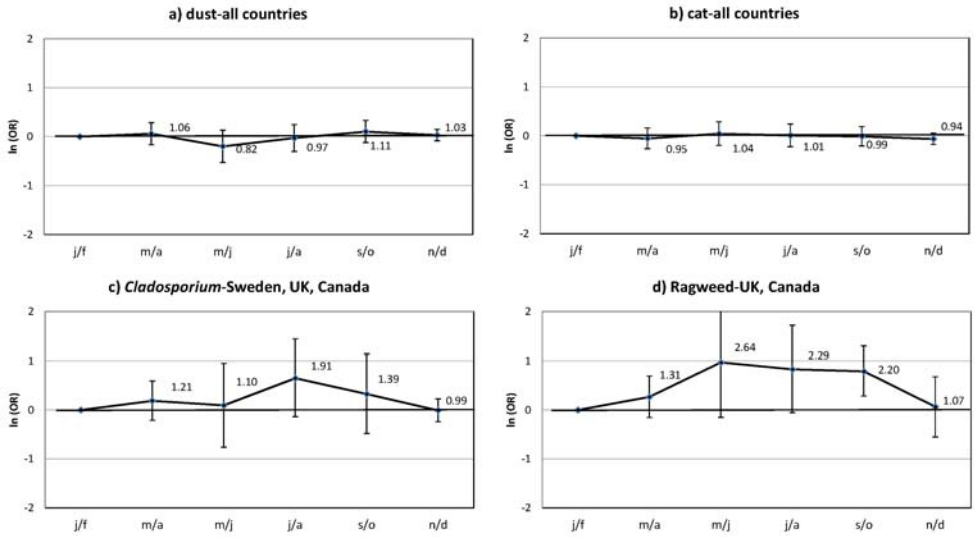
Table 2. The proportion of participants with information who reported their asthma attacks occurred at specific bi-monthly periods of the year and the adjusted odds ratio~ of reporting that asthma attacks usually occur in a given bi-monthly period by country (without consideration of sensitisation to aeroallergen)

country	% of participants reporting their asthma attacks occurred at specific bi-monthly periods of the year	OR: Jan/Feb	OR: March/April	Or: May/June	OR: July/August	Or: September/ October	OR: November/ December
Spain	86%	1.00	**1.54**	0.94	0.47	0.90	1.11
Italy	85%	1.00	1.54	**2.44**	1.10	0.81	0.81
France	70%	1.00	0.97	1.06	0.74	**0.67**	0.88
Switzerland	70%	1.00	**1.74**	**2.36**	1.18	1.07	0.97
Germany	76%	1.00	1.05	1.15	0.95	1.05	1.15
Belgium	67%	1.00	1.57	**2.35**	1.79	**2.05**	**1.91**
Netherlands	75%	1.00	1.00	**0.37**	**0.34**	0.85	1.09

Ireland	79%	1.00	0.35	0.44	0.82	0.74	1.11
UK	61%	1.00	**0.63**	0.84	0.97	**0.72**	1.00
Sweden	47%	1.00	1.09	**1.48**	1.04	0.91	1.09
Norway	50%	1.00	0.86	0.74	1.18	0.86	0.74
USA	72%	1.00	0.83	**2.38**	1.00	1.06	0.89
Canada	79%	1.00	**0.70**	0.81	0.80	0.79	0.91
New Zealand^	70%	1.00	**0.71**	**0.45**	**0.37**	**0.44**	1.00
Australia^	59%	1.00	**1.66**	1.07	0.77	0.59	0.81

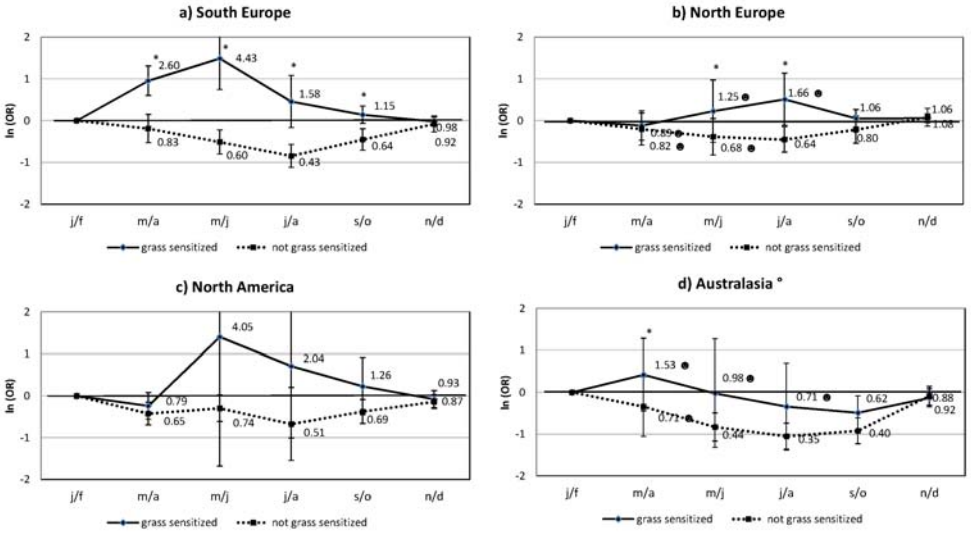
~OR: Odds ratio coefficient from GEE models controlling for repeated individual observations, gender and age. ^ 'bi-months' have been shifted 6 months forward to allow comparison with Northern hemisphere. In bold, statistically significant results, p-value<0.05.

Figure 1: The overall[+] adjusted odds ratio (95% CI) of asthma usually occurring in each bi-monthly period in those sensitised to house dust mite (2a), cat (2b), Cladosporium (2c) and ragweed (2d) (compared to those not sensitised to house dust mite and cat respectively)



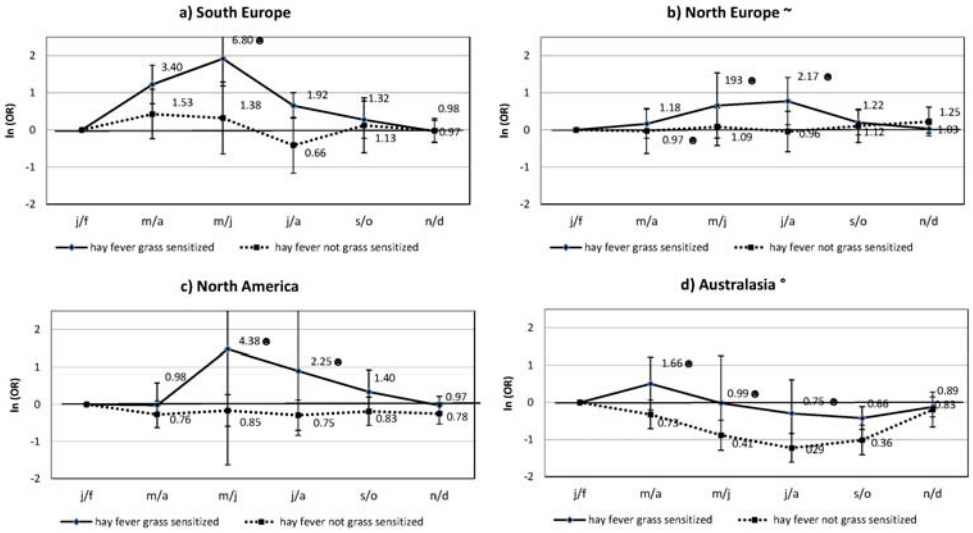
[+] from metanalysis of interaction coefficients from GEE models conducted within country controlling for repeated individual observations, main effect of relevant allergen and bi-monthly periods, gender, age and sensitization to dust mite (for cat) , cat (for house dust mite) and grass, birch and *Alternaria.*

Figure 2: The overall[+] adjusted odds ratio (error bars are 95% CI) of asthma usually occurring in each bi-monthly period in those sensitised to grass and in those not sensitised to grass



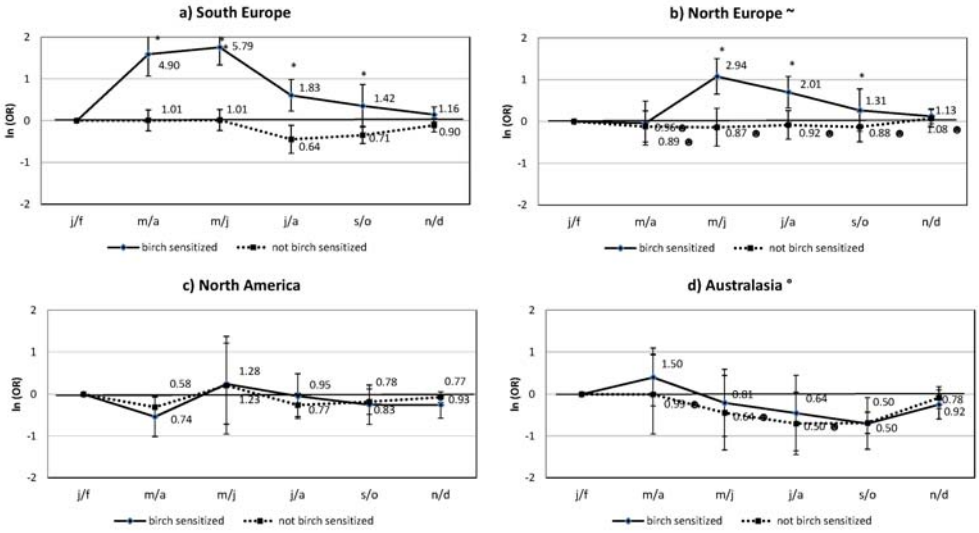
[+] from metanalysis of bi-monthly periods coefficients from GEE models conducted within country controlling for repeated individual observations, gender, age and sensitization to dust, cat, *Alternaria*, birch. ° 'bi-months' have been shifted 6 months forward to allow comparison with Northern hemisphere. ☻ p-heterogeneity<0.05. * p-value for interaction <0.05.

Figure 3: The overall[+] adjusted odds ratio (error bars are 95% CI) of asthma usually occurring in each bi-monthly period in those with reported hay fever sensitised and not sensitised to grass



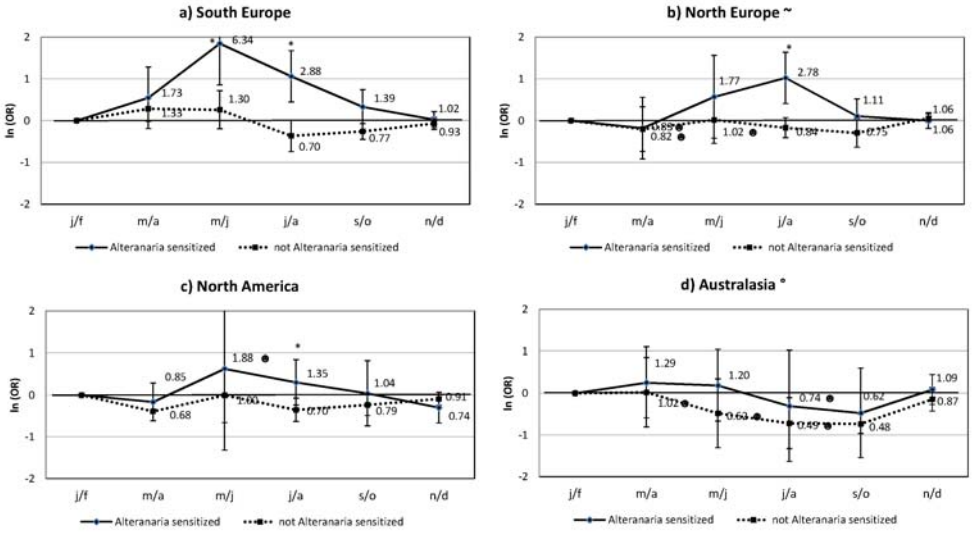
[+] from metanalysis of bi-monthly periods coefficients from GEE models conducted within country controlling for repeated individual observations, gender, age (only among subjects with sensitization to dust, cat, *Alternaria*, birch data). ° 'bi-months' have been shifted 6 months forward to allow comparison with Northern hemisphere. ☻ p-heterogeneity<0.05.

Figure 4: The overall[+] adjusted odds ratio (error bars are 95% CI) of asthma usually occurring in each bi-monthly period in those sensitised to birch and in those not sensitised to birch



[+] from metanalysis of bi-monthly periods coefficients from GEE models conducted within country controlling for repeated individual observations, gender, age and sensitization to grass, dust, cat, *Alternaria*.° 'bi-months' have been shifted 6 months forward to allow comparison with Northern hemisphere. ☻ p-heterogeneity<0.05. * p-value for interaction <0.05. ~Netherland and Ireland not included due to few asthmatics sensitized to birch producing lack of model convergence.

Figure 5: The overall[+] adjusted odds ratio (error bars are 95% CI) of asthma usually occurring in each bi-monthly period in those sensitised to *Alternaria* and in those not sensitised to *Alternaria*



+ from metanalysis of bi-monthly periods coefficients from GEE models conducted within country controlling for repeated individual observations, gender, age and sensitization to grass, dust, cat, birch. ° 'bi-months' have been shifted 6 months forward to allow comparison with Northern hemisphere. ☻ p-heterogeneity<0.05. * p-value for interaction <0.05. ~Netherland, Belgium, Ireland and Norway not included due to few asthmatics sensitized to *Alternaria* producing lack of model convergence.